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EFFECT OF TRAINING ON RAT SKELETAL MUSCLE MYOFIBRILLAR ATPASE, CALCIUM UPTAKE AND BINDING DURING DEVELOPMENT

by

© ANGELO N. BELCASTRO

A THESIS

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled: "Effect of Training on Rat Skeletal Muscle Myofibrillar ATPase, Calcium Uptake and Binding During Development", submitted by Angelo N. Belcastro in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physical Education.

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ABSTRACT

The intent of this study was to observe the effects of different training programs upon selected biochemical properties of young rats, in which the motor system is not fully matured. Young 10 day littermates were randomly assigned to 3 groups: Endurance (E), Sprint (S) and Control (C). Animals from all three groups were partitioned into either 21, 51 day exercising groups and 10 day controls. The E training consisted of continuous running for 60 min/day at 20m/min, 5% grade for 21 day animals while 51 day animals were progressed to 120 min/day at 40m/min, 15% grade. The 21 day S group performed 15 bouts/day (20 sec work/30 sec rest) at 30 m/min, 5% grade and progressed to 40 bouts/day at 80m/min, 15% grade at 51 days. The plantaris muscles of S animals were not altered at 21 and 51 days compared to C animals (all variables). Maturation showed increased myofibrillar ATPase activities and FSR parameters ($p \leq 0.05$) in the C animals. The 51 day E animals had lower ($p \leq 0.05$) myofibrillar ATPase values than 21 day E and 51 day C and S animals. The Ca^{++} binding ability increased with E training at 51 day ($p \leq 0.05$), while other FSR parameters did not change ($p \geq 0.05$). The developing soleus muscle of C rats had lower values than the S and E groups for each parameter except Ca^{++} uptake. The 21 and 51 day S rats had retained the myofibrillar ATPase activity present at 10 days. FSR parameters were not altered with S training. The myofibrillar ATPase activity, Ca^{++} uptake and FSR yield in the soleus were higher ($p \leq 0.05$) for 51 day E rats, than the C group. The Ca^{++} binding ability was not changed with E training. It is suggested that

function, especially E training may alter the normal developmental pattern of young rat skeletal muscle.

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INTRODUCTION

The identification of skeletal muscle fiber types by biochemical, histochemical and physiological techniques has led to the discovery that preferential recruitment of one type or another occurs during exercise. From glycogen depletion studies, Gollnick et al. (1974) found that the intensity and/or speed at which a muscle contracts will determine the type of muscle fiber to be predominantly recruited. At the cellular level this recruitment phenomenon appears to follow a logical pattern, since muscle fibers are metabolically either predominantly aerobic or anaerobic and have either slow or fast twitch contractile properties.

A great deal of literature is devoted to the classification of muscle fiber types, as well as their particular functional characteristics. Close (1974) in an extensive review of this literature has suggested that muscle fibers should be classifed according to biochemical, histochemical and physiological properties. Thus, the nomenclature employed in this study will be fast-twitch oxidative glycolytic (FOG), slow-twitch oxidative (SO), and fast-twitch glycolytic (FG) (Peter et al., 1972).

Characteristics of the three fiber types (Appendix A) have led to an increasing awareness of the specific changes occuring at the cellular level due to various training protocols. The adaptability of a muscle to an endurance training program is quite pronounced and specific as indicated by increases in concentrations of enzymes such as succinic acid dehydrogenase

(SDH), cytochrome c and other "oxidative" enzymes within the mitochondria (Holloszy et al., 1975; Molé et al., 1970, Baldwin et al., 1972; Barnard et al., 1970). The adaptations although specific to this particular energy supplying pathway, occur in all three fiber types. This indicates that the involvement of the different muscle fiber types in exercise is not a mutually exclusive phenomenon; rather they all appear to be utilized, with some more than others. For example, substrate availability, blood supply, muscle tension and joint angle, may affect the recruitment pattern of individual fibers during exercise. Studies with anaerobic or sprint type training, have shown that concentrations of phosphofructokinase (PFK) and lactic acid dehydrogenase (LDH) are increased due to the specific nature of the task (Karlsson et al., 1975; Thorstensson et al., 1975). The results associated with sprint training are not as conclusive as those reported for endurance work. Jobin (1977) observed no significant effect on glycolytic enzymes, while Hickson et al. (1976) reported a decrease in glycolytic enzyme concentrations following anaerobic or sprint type training. The controversy associated with sprint training may be related in part to methodology (e.g. muscle assayed), the neural activity required during sprint training or the already present high activity of glycolytic enzymes.

The relative importance of neural activity on muscle fiber characteristics has been demonstrated by cross-innervation studies, where FG and/or FOG fibers have been transformed into

SO fibers (Buller, Eccles and Eccles, 1960, a, b). The changes are not only manifest in enzyme concentrations, but also in contractile properties (e.g., time to contraction, and one half relaxation time). The effects upon the contractile properties suggest a change in contractile proteins directly related to the neural activity imposed upon the muscle. Changes in myosin ATPase activity and light chain components have also been reported after cross-innervation studies, particularly in young animals (Pette et al., 1976; Gutmann et al., 1974; Sreter et al., 1974).

Alterations of contractile characteristics have not only been observed with cross-innervation and post-natal development, but also the dependence on muscle function has been considered. Since soleus muscle serves a primary role in postural or anti-gravity function, it has been postulated that the disappearance with growth of FG and FOG fibers, present at birth in soleus, may be attributed to their minimal involvement with postural function. The neural activity accompanying different types of function may be the primary reason for influencing characteristics of a particular fiber type (Pelloni-Mueller et al., 1976; Lewis et al., 1974). In an attempt to further observe this "function-fiber type" theory, exercise programs of varying intensities and duration have been employed. The contractile properties (e.g. time to peak tension, half relaxation, twitch tension and twitch to tetanus ratio) of muscle from treadmill trained animals did not differ significantly from sedentary controls (Fitts et al., 1973; Barnard et al., 1970).

Whereas, intense swimming resulted in 9% and 12% decrease in time to peak tension and half relaxation time respectively for rat EDL muscle (Gutmann and Hajek, 1971). In terms of myofibrillar, or myosin ATPase activities, the data are also controversial. Increased myofibrillar ATPase activities in rat EDL and gastrocnemius muscles after intense swimming programs were reported by a number of investigators (Syrovy et al., 1972; Gutmann and Hajek, 1971; Wilkerson and Evonuk, 1971). Endurance type treadmill running produced no significant changes in the myofibrillar ATPase of rat FG muscle, but showed a significant decrease for FOG muscle and a significant increase in SO muscle (Baldwin et al., 1975). Thus, it appears that enhanced muscle function via exercise, which also requires increased, specific nerve traffic to a muscle, may be important in producing changes in myofibrillar ATPase activity.

In summary, the effects of both endurance and sprint type training and the associated neural activity on the contractile apparatus (e.g. myofibrillar ATPase, and Ca^{++} uptake by the sarcoplasmic reticulum) is unclear. Training-induced enzymatic changes, increases the muscles potential to generate energy from a particular pathway. Whether or not the structural and/or functional properties of the muscle adapt due to training, thus enabling a muscle and ultimately an athlete to enhance the contractile mechanism's potential to generate a specific type of contraction is also unclear. Since the contractile properties exhibited by a particular fiber type are related in part, to

structure, it would seem that training would alter this component as well as the metabolic features. Since changes may be more readily elicited during development the intent of this study is to observe and report the effects of two different training regimens upon the selected properties of young animals, in which the motor system is not fully mature.

The following dependent variables were used to study developing muscle adaptations to sprint and endurance exercise.

Biochemical Properties:

1. Mg^{++} - activated myofibrillar ATPase - This has been shown to correlate highly with the speed of contraction and represents the capability of myofibrillar protein to hydrolyze ATP.
2. Fragmented sarcoplasmic reticulum (FSR) Ca^{++} uptake and binding - The FSR has two mechanisms for removing Ca^{++} from the sarcoplasm: One which rapidly binds Ca^{++} to a protein on the membrane and is termed Ca^{++} binding and another which deposits Ca^{++} in the matrix of the SR and is slow (Ca^{++} uptake). Both transport systems are ATP dependent.

Histochemical Properties:

Fibre types were classified with cryocut sections (10 u) mounted on cover slips and stained to illustrate the following:

1. Contractile potential - myofibrillar ATPase stains were

incubated at pH 9.4 after pre-incubation at pH 10.4 for 15 minutes.

2. Oxidative potential - NADH diaphorase stain was used to qualitatively assess the reduction of NADH by the muscle fibres.

METHODOLOGY

1. Animal Care

Woodlyn-Wistar[1] rats (175) at 10 days of age were initially assigned to the study. 121 of these animals were assigned to the 10 days of age group to permit sufficient tissue for the subsequent biochemical analyses. The remaining 54 animals were then randomly assigned to one of 6 remaining groups: sprint (S) 21 day, S 51 day control (C) 21 day, C 51 day, endurance (E) 21 day and E 51 day. Animals were killed at 21 and 51 days for convenience and for comparisons of maturing and adult animals. Due to injury, disease or failure to run, the attrition average for all groups was 22%. Individual group attrition values are in Appendix H.

The animals were housed with their mothers at all times, except for training purposes, until they were weaned (21 days). All animals were weighed at 10 days after birth and once a week until death. The C group was handled as often as the experimental groups (E and S) and removed from their mothers for a comparable period of time. After handling or training of young animals, they were placed in a clean cage and sprinkled with bedding from their previous cage. This was done to prevent maternal cannibalism. The mother was re-united with her young after each animal had completed the prescribed treatment.

The animals were fed a regular diet of laboratory chow

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[1]From Woodlyn Farms Ltd., R.R. 3, Guelph, Ontario

and water, ad libitum. Also, all animals were subjected to a controlled environment with a temperature of 20±1°C and humidity of 35±3% and maintained on a day-night cycle of 12 hours (e.g., light period from 8:00 a.m. to 8:00 p.m.).

2. Training Program

The training program for the E group consisted of progressive treadmill running for six weeks at a final speed of 40 m/min, 15% grade, 120 min./day, 4 days/week. The S group also completed a progressive program at 40 bouts/day at a work: rest ratio of 20:30 seconds up to a speed of 80 m/min. and a grade of 15% for 4 days/week. Specific details of the program progressions are outlined in Table 2:1.

The experimental design is as illustrated in figure 2:1. The killing was done by decapitation prior to the training (10 days) after 8 training sessions (21 days) and after 24 training sessions (51 days). A period of 24 hours and 72 hours was allowed to elapse prior to sacrificing the animals for the 21 day and 51 day groups, respectively. Only 24 hours was permitted to expire following the last training session in the 21 day animals because of the dramatic developmental changes happening during this period.

TABLE 2:1

Training Protocol

Endurance (E)

WEEK	SESSIONS	SPEED (m/min)	TIME OR BOUTS	GRADE %
1	4	5	30 min/day	5
2	2	5	60 min/day	5
	2	20	60 min/day	5
3	2	30	60 min/day	15
	2	30	120 min/day	15
4	2	40	60 min/day	15
	2	40	90 min/day	15
5	4	40	90 min/day	15
6	4	40	120 min/day	15

Sprint (S)

1	2	10	20 min/day	5
	2	20	15 bouts/day	5
2	2	20	20 bouts/day	5
	2	30	15 bouts/day	5
3	1	30	30 bouts/day	15
	1	40	30 bouts/day	15
	2	60	20 bouts/day	15
4	2	60	30 bouts/day	15
	2	70	20 bouts/day	15
5	2	70	30 bouts/day	15
	2	80	30 bouts/day	15
6	4	80	40 bouts/day	15

GROUPS	DAYS		
	10	21	51
Control	X	X	X
Sprint	X	X	X
Endurance	X	X	X

Figure 2:1 Experimental Design Including Sacrifice Sequence (X).

3. Tissue Sampling

Following decapitation, the animals were exsanguinated, and the soleus and plantaris muscles excised from both legs. Once removed, the muscles were weighed, frozen in isopentane cooled by liquid nitrogen and stored at -50°C, for determination of Mg^{++} - activated myofibrillar ATPase activity, and Ca^{++} uptake by fragmented sarcoplasmic reticulum. Since the amount of tissue required for analyses is relatively large, muscles from 10 day animals were pooled as described in subsequent sections.

4. Biochemical Analysis

(Details in Appendix C)

Pooled muscles (3 animals) were homogenized in a borate-KCl buffer (pH 7.1): 0.039M sodium borate, 0.025 M KCl, 5mM EDTA (Perry and Corsi, 1958). An aliquot (1.0 ml) was taken for determination of total protein by the method of Lowry et al. (1951). The homogenate was centrifuged at 800 x g for 15 minutes and the supernatant discarded. The muscle pellet at 800 x g was resuspended and recentrifuged in a borate-KCl buffer for the isolation and separation of the myofibril fraction. Small aliquots (0.5 ml) were taken for the determination of myofibrillar protein.

The separated myofibrils were prepared for myofibrillar ATPase determination by double washing with: 50mM Tris, 5mM $MgCl_2$,

100mM KCl and 0.1% Triton X-100 (pH 7.1). The washed myofibrils were suspended in 150mM KCl and 50mM Tris-HCl (pH 7.4). The Mg^{++} activated ATPase activity was carried out as described by Baldwin et al. (1975) (Appendix C). The reaction mixture: 7mM KCl, 0.01mM $CaCl_2$, 1mM $MgCl_2$ and 20 mM Tris-HCl (pH 7.4) was pre-incubated at 30°C for 5 minutes, with the myofibril solution. After this incubation 1mM ATP was added and the sample mixed thoroughly and allowed to incubate for another 5 minutes at 30°C. Cessation of the reaction was carried out by rapid addition of 0.1 volume perchloric acid and neutralized by 7 volumes of KOH. The samples were centrifuged at 1000 x g for 10 minutes to precipate protein and the supernatant (0.5 ml) was used for the determination of Pi (umoles Pi/mg/min) (Gawehn, 1974).

5. Calcium Uptake and Binding

(Details in Appendix C)

Fragmented sarcoplasmic reticulum vesicles from 200 mg of pooled muscle samples (2-25 animals), were prepared in 10mM sodium bicarbonate and 5mM sodium azide (pH 7.0) buffer and suspended in 50mM KCl and 20mM Tris-maleate (pH 6.8) (Harigaya and Schwartz, 1969). The Ca^{++} - binding (nmoles Ca^{++}/mg protein) was determined after 200 ug of vesicle protein/ml was incubated at 37°C for 1 min. in a mixture containing: (in mM) KCl 100; $MgCl_2$ 10, Tris-maleate (pH 6.8) 20; Tris-ATP2; and 10 uM $CaCl_2$ with 0.01 uCi/ml of aqueous $^{45}Ca^{++}$. FSR related parameters were obtained for 10 and 51 day

animals and not 21 day animals due to the small amount of total tissue (approximately 250 mg) harvested.

Ca^{++} - uptake (umoles Ca^{++} /mg protein) was determined in a similar reaction mixture with the addition of 5mM sodium oxalate and a higher (100 uM) $CaCl_2$ content after incubation at 37°C for 30 min. (Harigaya and Schwartz, 1969).

Following the respective incubation periods for Ca^{++} - binding and Ca^{++} - uptake, an aliquot (0.8 ml) of the sample was filtered (Millipore Filter Ha-0.45 u) against a vacuum and the filtrate used for $^{45}Ca^{++}$ determination in 5 ml of Aquasol (New England Nuclear). The calculation of Ca^{++} activities by sample filtrates were accomplished by comparing cpm values against a standard curve (cpm vs Ca^{++} in umoles/ml).

6. Histochemical Analysis

After having excised the muscles, a portion was removed and frozen in isopentane, cooled in liquid nitrogen and the tissue was frozen in an embedding medium so that freezing artifact would be minimized.

Sections (10u) were stained for NADH-diaphorase (Dubowitz and Brooke, 1973) and myofibrillar ATPase preincubated at a pH of 10.4 (Guth and Samaha, 1969).

7. Statistical Analysis

Mg^{++} - activated myofibrillar ATPase activities (umoles Pi/mg/min) and the rate of Ca^{++} - binding (nmoles Ca^{++}/mg protein) and uptake (umoles Ca^{++}/mg protein) before and after training in each of the groups were tested for significance using a two-way analysis of variance (Winer, 1971). Homogeneity of variance between the groups was tested by the Chi Square method and significant values from the two-way ANOVA were tested using Student-Newman-Keuls post hoc test (Morehouse et al., 1975) at a p level of 0.05.

RESULTS

1. Training Program

The training program used in this study is summarized in Table 2:1. The endurance (E) and sprint (S) programs were successful as measured by performance tests 24 and 72 hours prior to sacrificing the 21 and 51 day animals respectively. The 21 day S group completed 43.7±0.6 bouts of exercise (work: rest ratio 20:30 sec) at 40 m/min., 15% grade and the 51 day group performed 110.3±4.1 bouts at 80 m/min., 15% grade. The E groups at 21 and 51 days of training had performance times of 214.3±35.7 min. and 63.0±0.2 min., at 20 and 40 m/min., 15% grade, respectively.

2. Body and Muscle Weights

The 51 day S and E groups had body weights of 178.1± 15.6g and 184.3±15.7g respectively. These are significantly ($p \leq 0.05$) lower than the C group (227.1±6.5g). Muscle weights for both the soleus and plantaris were not significantly different ($p \geq 0.05$) between groups at either 21 or 51 days.

At 51 days all the body and muscle weights had become heavier as the animals matured ($p \leq 0.05$) (Table 3:1). However, only the body weights and not the muscle weights at 21 days were greater than those observed for the 10 day animals ($p \leq 0.05$).

TABLE 3:1

Body and Muscle weights of Control, Sprint and Endurance Trained Animals ($\bar{X} \pm$ SEM), at 10, 21 and 51 days.

	10 days	21 days	51 days
Body Weights (g):			
Control	19.6 ± 2.4(5)	53.4 ± 2.3[c](5)	227.1 ± 6.5[a,b](7)
Sprint	18.5 ± 0.4(6)	55.8 ± 5.0[c](6)	178.1 ± 15.5[a,b,e](6)
Endurance	22.4 ± 4.6(7)	56.1 ± 4.4[c](6)	184.3 ± 15.7[a,b,f](6)
Soleus (mg):			
Control	8.4 ± 0.8	21.4 ± 1.6	85.7 ± 6.8[a,b]
Sprint	5.8 ± 1.0	21.2 ± 2.8	79.0 ± 10.1[a,b]
Endurance	9.1 ± 2.4	23.8 ± 2.5	81.3 ± 10.3[a,b]
Plantaris (mg):			
Control	10.1 ± 1.7	30.6 ± 2.7	168.1 ± 10.1[a,b]
Sprint	8.2 ± 0.8	31.7 ± 2.7	145.7 ± 8.9[a,b,e]
Endurance	12.3 ± 4.1	33.5 ± 2.9	130.3 ± 15.4[a,b,f]

Statistical Comparison among groups, p 0.05

N. in brackets

a. 51d vs 21d

b. 51d vs 10d

c. 21d vs 10d

d. S vs E

e. S vs C

f. E vs C

3. Plantaris Muscle

At each age, the total protein concentration increased significantly ($p \leq 0.05$) in the C group, with values at 10, 21 and 51 days of 171.1 $\pm$ 1.8, 233.5 $\pm$ 5.7 and 298.2 $\pm$ 20.1 mg/g, respectively. The two training programs did not follow the same pattern of total protein increases with age, as observed for the C group. The training programs resulted in higher total protein concentrations at 21 days with the E group (319.2 $\pm$ 20.8 mg/g) having higher total protein concentrations than the C group at the same age. The 21 day S group was not different ($p \geq 0.05$) from either the C or E groups (Table 3:2). At 51 days the protein concentrations had decreased to 278.9 $\pm$ 16.0 mg.g and 265.6 $\pm$ 24.1 for the E and S groups, respectively ($p \leq 0.05$). The differences between all groups at this age were not significant ($p \geq 0.05$). No differences in myofibrillar protein concentration occurred at any age or between any of the training programs ($p \geq 0.05$) (Table 3:2).

Mg^{++} - activated myofibrillar ATPase activities were higher ($p \leq 0.05$) at 21 days as compared to the 10 day values for all groups (figure 3:1). The 21 day training programs had no effect on the Mg^{++} - ATPase activities in the plantaris, the values for the C, S and E groups were 0.720 $\pm$ 0.037, 0.748 $\pm$ 0.019 and 0.701 $\pm$ 0.028 umoles Pi/mg/min, respectively ($p \geq 0.05$). At 51 days, there was a significantly lower Mg^{++} - activated ATPase activity in the E group (0.585 $\pm$ 0.015 umoles Pi/mg/min), when compared to the 21

TABLE 3:2

Total and Myofibrillar protein concentrations (mg/g) of Plantaris Muscles from Control, Sprint and Endurance Trained Animals ($\bar{X} \pm$ SEM).

TOTAL	10 days	21 days	51 days
Control	171.1 ± 1.7	233.5 ± 5.7[b] (5)	298.2 ± 20.1[a,c] (7)
Sprint	173.7 ± 1.4 (6)	309.2 ± 2.0[b] (6)	278.9 ± 16.0[a,c] (5)
Endurance	182.0 ± 1.3 (5)	319.2 ± 20.7[b,d]	265.6 ± 24.1[a,c] (6)
MYOFIBRIL			
Control	136.0 ± 1.6	162.8 ± 3.9	177.1 ± 16.8
Sprint	170.3 ± 1.5	168.7 ± 10.6	178.9 ± 17.8
Endurance	154.7 ± 1.3	167.5 ± 18.4	181.8 ± 14.2

Statistical Comparison among groups, p 0.05

a. 51 vs 10d
b. 21 vs 10d
c. 51 vs 21d
d. E vs C
e. S vs C
f. E vs S

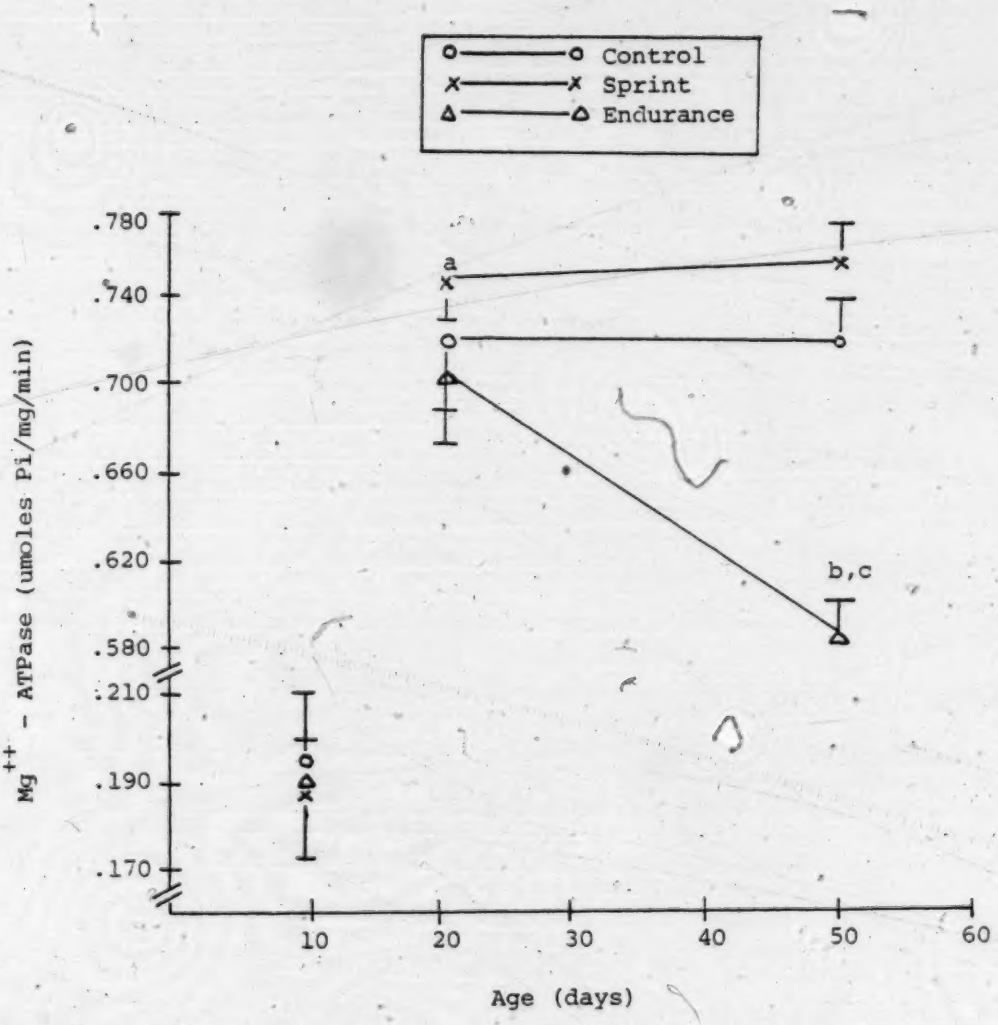

Figure 3:1 Mg^{++}-activated Myofibrillar ATPase Activities of Plantaris Muscle from Sprint and Endurance Trained Rats at 10, 21 and 51 days.

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a = 10d vs 21d (p 0.05) b = 21d vs 51d (p 0.05) C = E vs S,C, (p 0.05)

day value (0.701 $\pm$ 0.028 umoles Pi/mg/min) ($p \leq 0.05$). The 51 day E group activity was 22% lower than the S group (0.755 $\pm$ 0.016 umoles Pi/mg/min) and 19% lower than the C group (0.719 $\pm$ 0.011 umoles Pi/mg/min) ($p \leq 0.05$).

The yield of fragmented sarcoplasmic reticulum (FSR) protein was greater ($p \leq 0.05$) as the animals matured from 10 days to 51 days (figure 3:2). The FSR protein yields of 6.0 $\pm$ 0.3 mg/g and 6.8 $\pm$ 0.6 mg/g for the 51 day S and E training groups respectively, were not different than the value obtained (6.9 $\pm$ 0.6 mg/g) for the C group ($p \geq 0.05$). A similar phenomenon occurred for the calcium uptake activity of the plantaris muscle. Maturation resulted in significantly elevated calcium uptake values in the 51 day, C group (0.164 $\pm$ 0.004 umoles Ca^{++}/mg protein) as compared to the 10 day C group (0.149 $\pm$ 0.003 umoles Ca^{++}/mg protein). No significant differences were found as a consequence of either of the training programs (figure 3:3) ($p \geq 0.05$). The ATP - dependent calcium binding ability of the plantaris muscle was increased due to maturation (figure 3:4) ($p \leq 0.05$). The sprint training program did not effectively alter calcium binding when compared to the control group at 51 days. The E training group had increased the calcium binding ability by 23% over the C group, to a value of 26.1 $\pm$ 3.4 nmoles Ca^{++}/mg protein ($p \leq 0.05$) (figure 3:4).

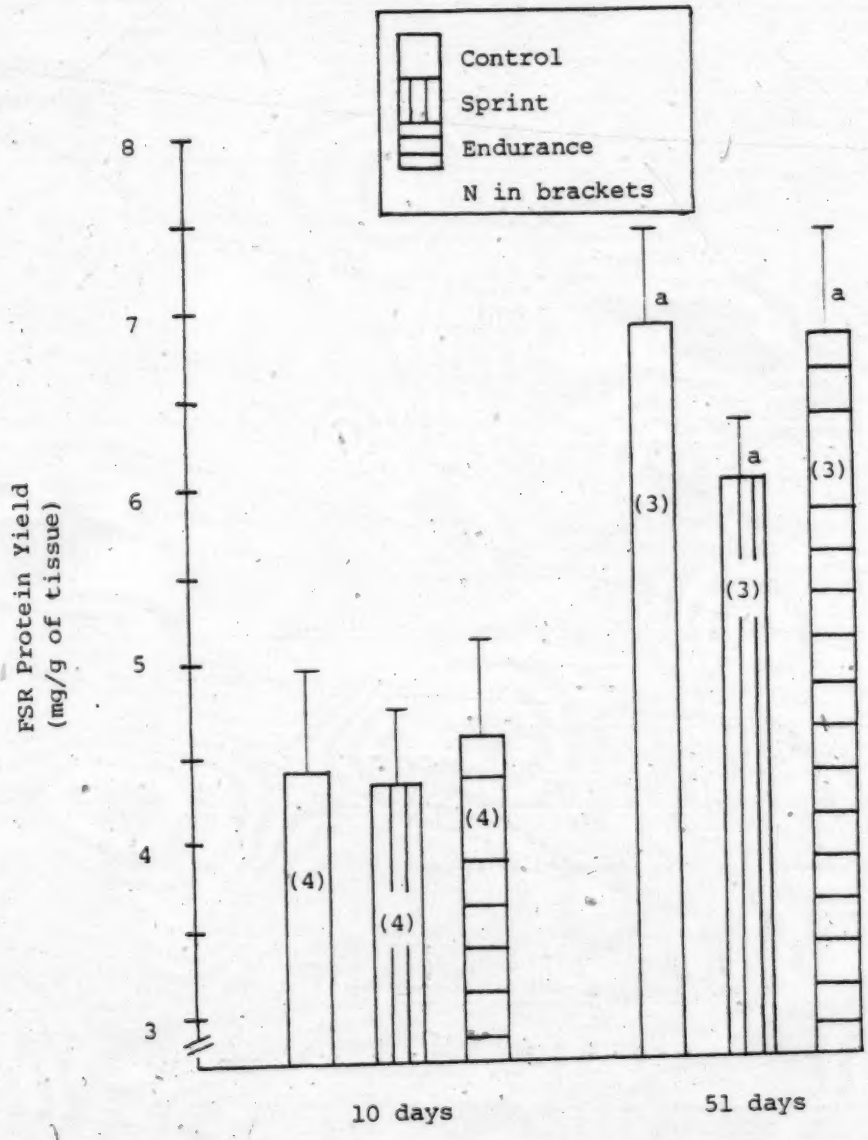

Figure 3:2 FSR Protein Yield of Pooled (2-25 animals) Plantaris Muscle ($\bar{X} \pm$ SEM). In order to make up an N of 1 for the 10 day animals, the muscle of 25 animals had to be pooled. For the 51 day animals the muscles of 3 animals were pooled to give the N of 1.

a 10d vs 51d ($p \leq 0.05$)

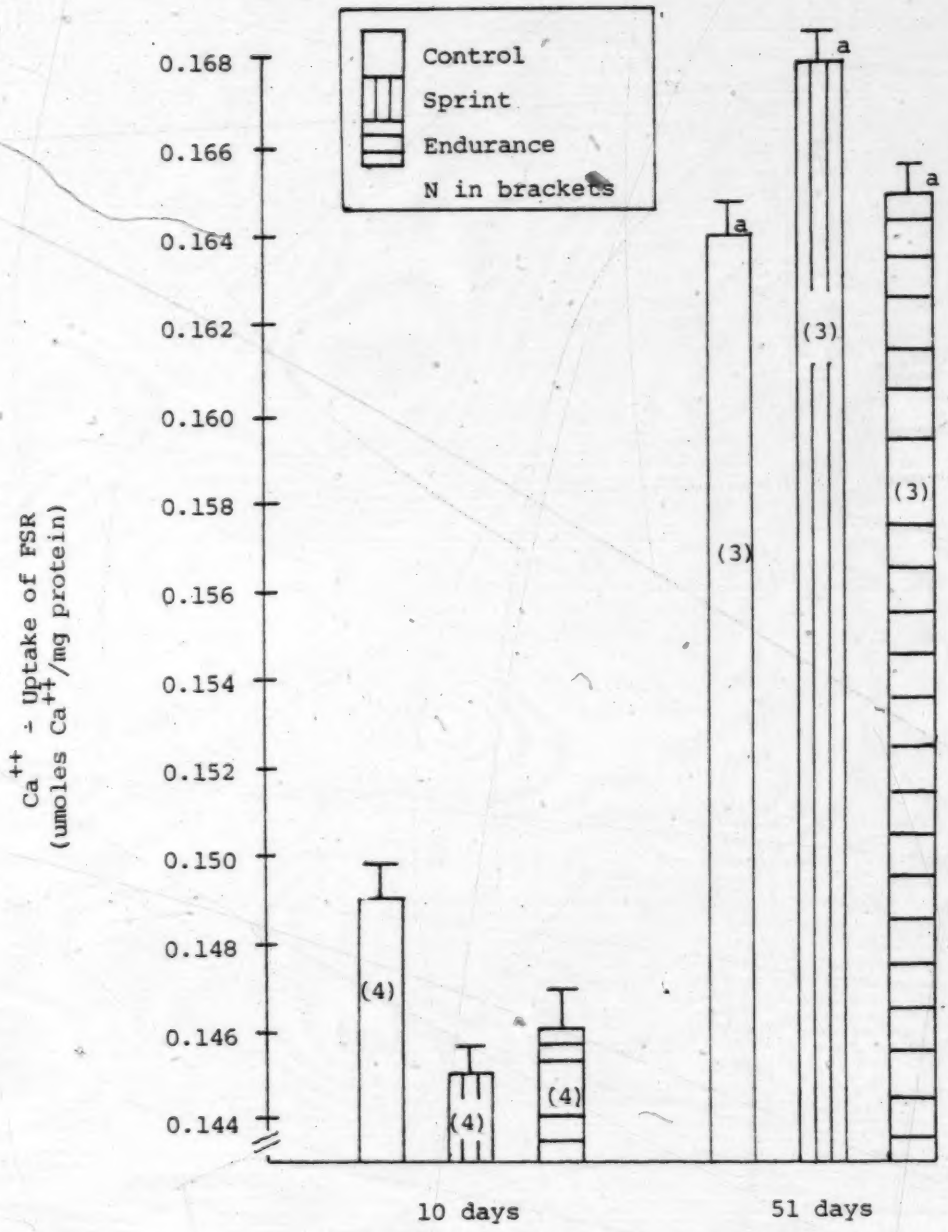

Figure 3:3 Ca^{++} - Uptake of Pooled (2-25 animals) Plantaris Muscle ($\bar{X} \pm$ SEM). In order to make up an N of 1 for the 10 day animals, the muscle of 25 animals had to be pooled. For the 51 day animals the muscles of 3 animals were pooled to give the N of 1.

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a 10d vs 51d ($p \leq 0.05$)

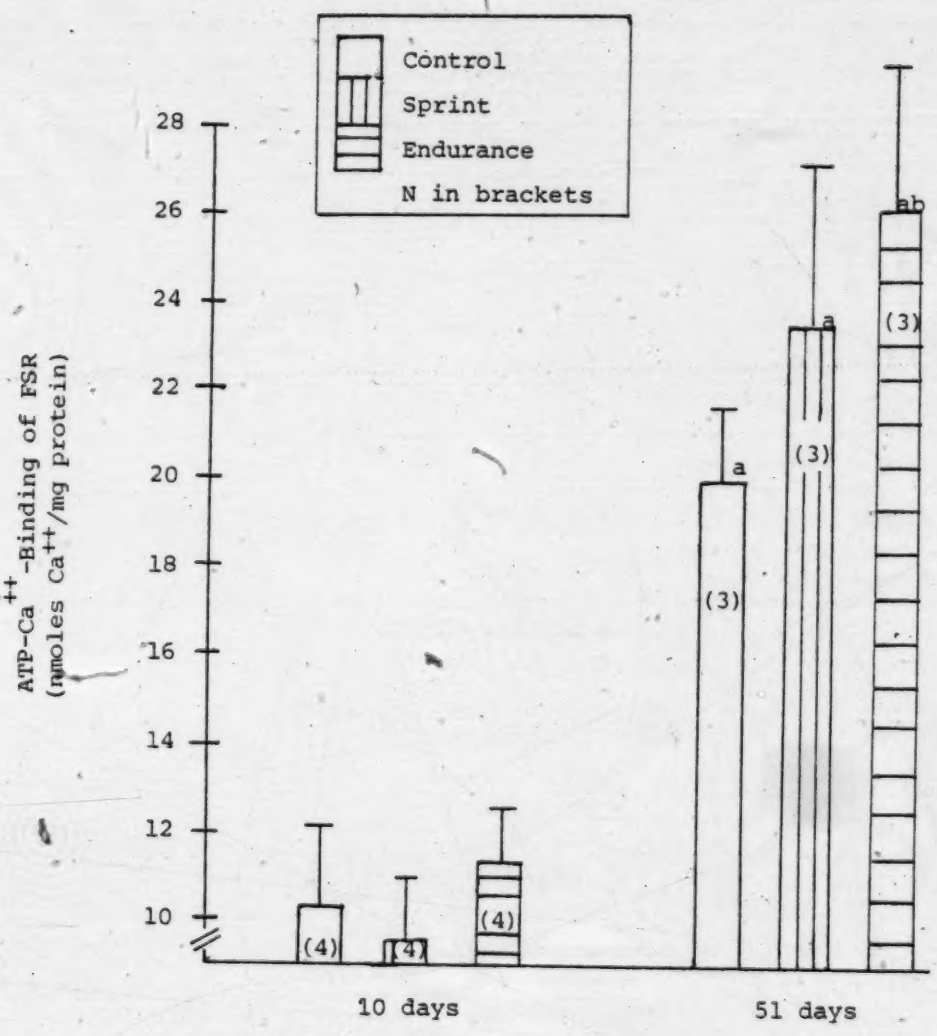

Figure 3:4 ATP-Ca^{++}-Binding of Pooled (2-25 animals) Plantaris Muscle ($\bar{X} \pm$ SEM). In order to make up an N of 1 for the 10 day animals, the muscle of 25 animals had to be pooled. For the 51 day animals the muscles of 3 animals were pooled to give the N of 1.

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a 10d vs 51d ($p \leq 0.05$)

b C vs E ($p \leq 0.05$)

4. Soleus Muscle

At each age the protein concentration of the C group was not significantly different due to maturation. The S and E groups at 21 and 51 days were not altered ($p \geq 0.05$) as a consequence of exercise (Table 3:3).

The myofibrillar protein concentration was not altered due to maturation for the C group ($p \geq 0.05$) (Table 3:3). At 21 days, the myofibrillar protein concentration for the E training group was not different than the C animals ($p \geq 0.05$). The S group had an elevated protein concentration (226.3 $\pm$ 3.4 mg/g) at 21 days which was significantly greater than the C group (124.7 $\pm$ 26.3 mg/g) ($p \leq 0.05$), but not different from the E group (143.5 $\pm$ 19.5 mg/g) ($p \geq 0.05$). No alterations for either the S or E groups at 51 days were observed when compared to the C group ($p \geq 0.05$) (Table 3:3).

The Mg^{++} - activated myofibrillar ATPase activities for the C group at 21 and 51 days (0.193 $\pm$ 0.029 and 0.172 $\pm$ 0.031 umoles Pi/mg/min) were reduced as a result of development, when compared to 10 day activities (0.312 $\pm$ 0.005 umoles Pi/mg/min) ($p \leq 0.05$). In contrast, the 51 day E group value was greater than the 10 day value ($p \leq 0.05$) (figure 3:5). The E group at 21 and 51 days (0.323 $\pm$ 0.008 and 0.378 $\pm$ 0.009 umoles Pi/mg/min) were greater than the C group at 21 day (0.193 $\pm$ 0.029 umoles Pi/mg/min) and 51 day (0.172 $\pm$ 0.031 umoles Pi/mg/min) ($p \leq 0.05$). The S group, Mg^{++}

TABLE 3:3

Total and Myofibrillar protein concentrations (mg/g) of Soleus Muscles from Control, Sprint and Endurance Trained Animals ($\bar{X} \pm$ SEM).

TOTAL	10 days	21 days	51 days
Control	237.1 ± 1.5 (6)	302.8 ± 47.1 (5)	244.3 ± 46.0 (7)
Sprint	242.6 ± 2.2 (6)	242.2 ± 3.7 (6)	282.2 ± 12.6 (6)
Endurance	271.1 ± 2.5 (6)	209.4 ± 1.3 (7)	224.5 ± 11.7 (6)
MYOFIBRIL*			
Control		124.7 ± 26.3 (4)	184.0 ± 21.3 (5)
Sprint		226.3 ± 3.4[e] (3)	189.2 ± 17.1 (5)
Endurance		143.5 ± 19.6 (3)	180.8 ± 2.9 (3)

Statistical Comparison among groups, p 0.05

a. 51 vs 10d
b. 21 vs 10d
c. 51 vs 21d
d. E vs C
e. S vs C
f. E vs S

* Missing data was due to small accident in laboratory.

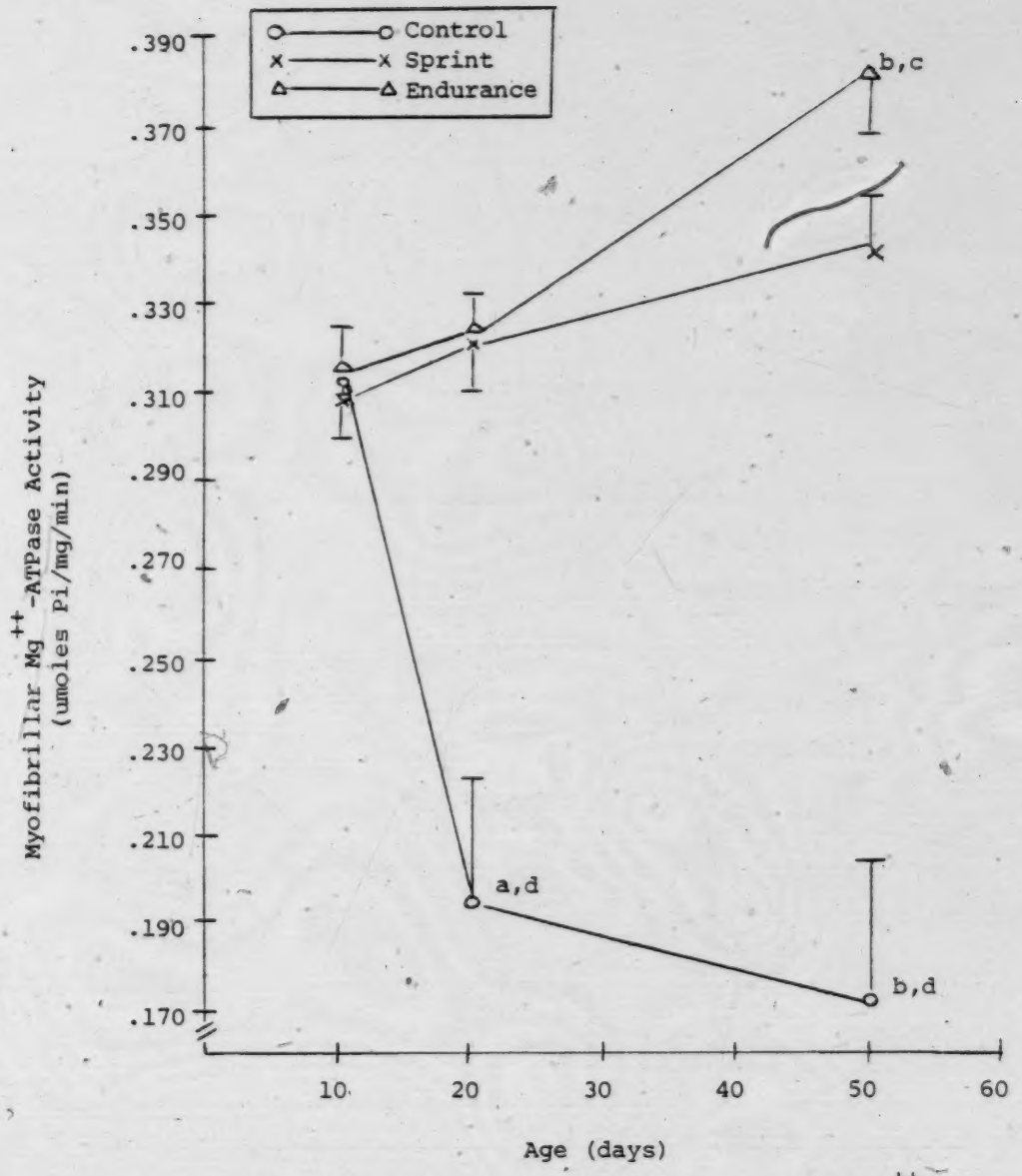

Figure 3:5 Effect of Endurance and Sprint Training on Mg^{++}-activated Myofibrillar ATPase Activities of 10, 21 and 51 day, Rat Soleus Muscle.

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a 10d vs 21d ($p \leq 0.05$)
b 10d vs 51d ($p \leq 0.05$)
c 21d vs 51d ($p \leq 0.05$)
d C vs S,E, ($p \leq 0.05$)

- activated myofibrillar ATPase activities were 39% and 49% greater at 21 and 51 days, when compared to the C group at the same ages ($p \leq 0.05$).

The FSR protein yield at 10 days for the C, S and E groups were not different ($p \geq 0.05$) (figure 3:6). At 51 days, there was a decrease in the amount of FSR protein recovered from the soleus muscle of both control and sprint trained rats. The C values had decreased to 5.7 $\pm$ 0.6 mg/g from 8.4 $\pm$ 0.6 mg/g and the S group had decreased to 5.8 $\pm$ 0.2 mg/g from 8.3 $\pm$ 0.6 mg/g ($p \leq 0.05$). The 51 day, E group (7.0 $\pm$ 0.3 mg/g) had not decreased from the 10 day, E group (7.9 $\pm$ 0.7 mg/g) ($p \geq 0.05$). At 51 days, none of the training programs resulted in significant differences compared to the C group (figure 3:6).

The ATP - dependent calcium binding abilities of the C, S and E groups were not different from each other at 10 or 51 days ($p \geq 0.05$) (figure 3:7). The maturation process resulted in a lower calcium binding capability of the older 51 day control animals when compared to the 10 day group. The same pattern was shown with both exercise conditions.

The calcium uptake of FSR protein for control and sprint soleus muscles were not different when the 10 day and 51 day groups were compared ($p \geq 0.05$) (figure 3:8). In contrast, the E group at 51 days (0.159 $\pm$ 0.006 umoles Ca^{++}/mg protein) ($p \leq 0.05$) had a greater calcium uptake than the 51 day S group (figure 3:8).

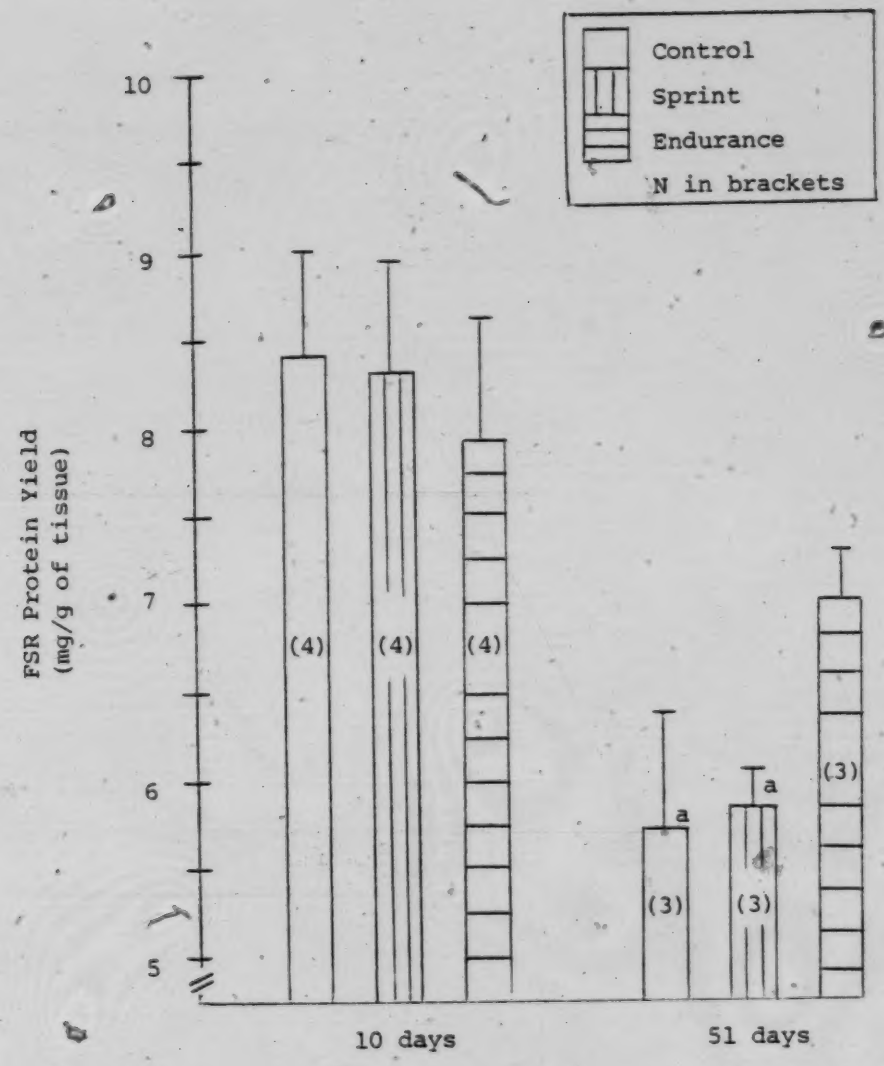

Figure 3:6 FSR Protein Yield of Pooled (2-25 animals) Soleus Muscle ($\bar{X} \pm$ SEM). In order to make up an N of 1 for the 10 day animals, the muscle of 25 animals had to be pooled. For the 51 day animals the muscles of 3 animals were pooled to give the N of 1.

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a 10d vs 51d ($p \leq 0.05$)

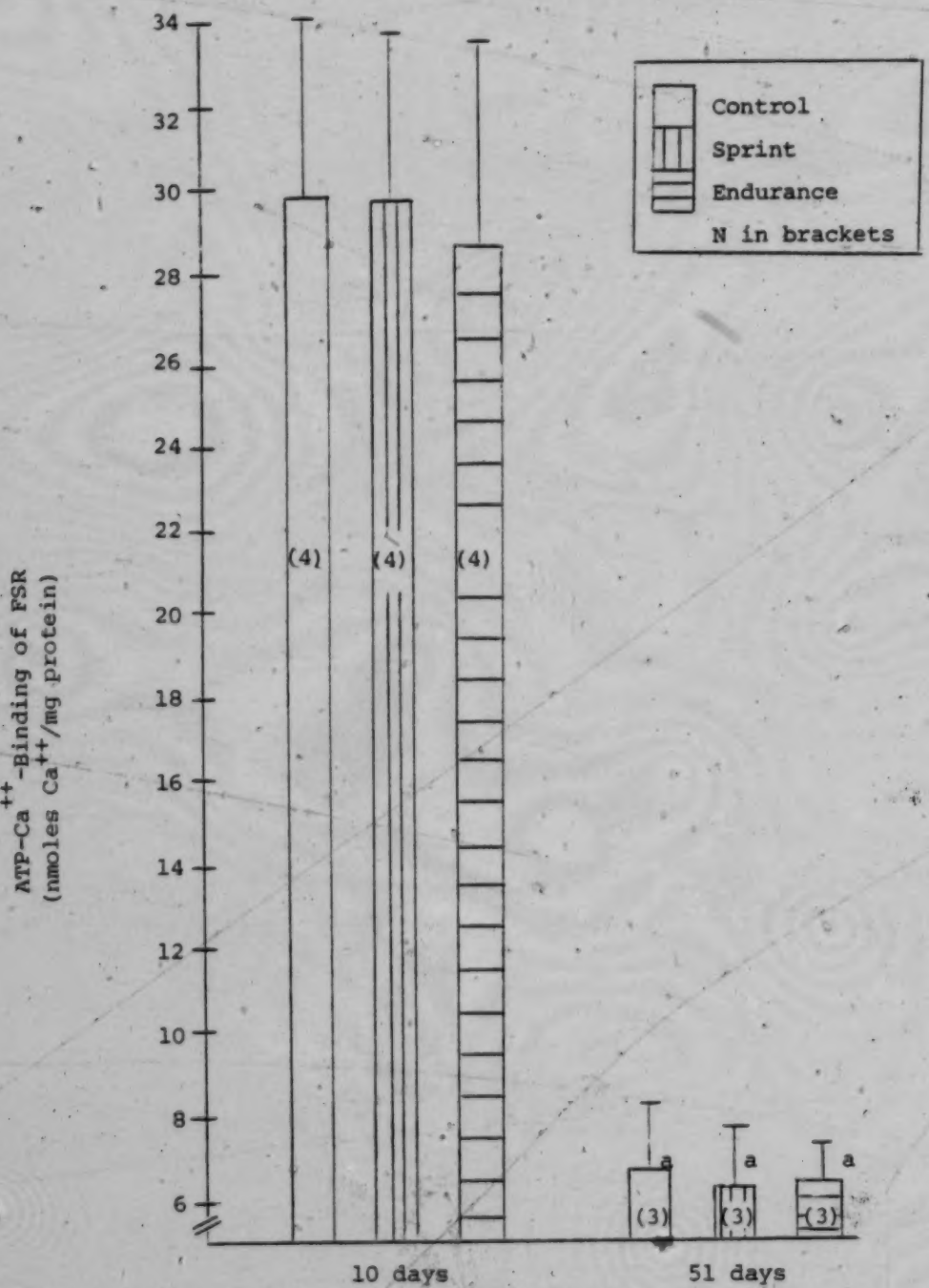

Figure 3:7 ATP-Ca^{++}-Binding of Pooled (2-25 animals) Soleus Muscle ($\bar{X} \pm$ SEM). In order to make up an N of 1 for the 10 day animals, the muscle of 25 animals had to be pooled. For the 51 day animals the muscles of 3 animals were pooled to give the N of 1.

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a 10d vs 51d ($p \leq 0.05$)

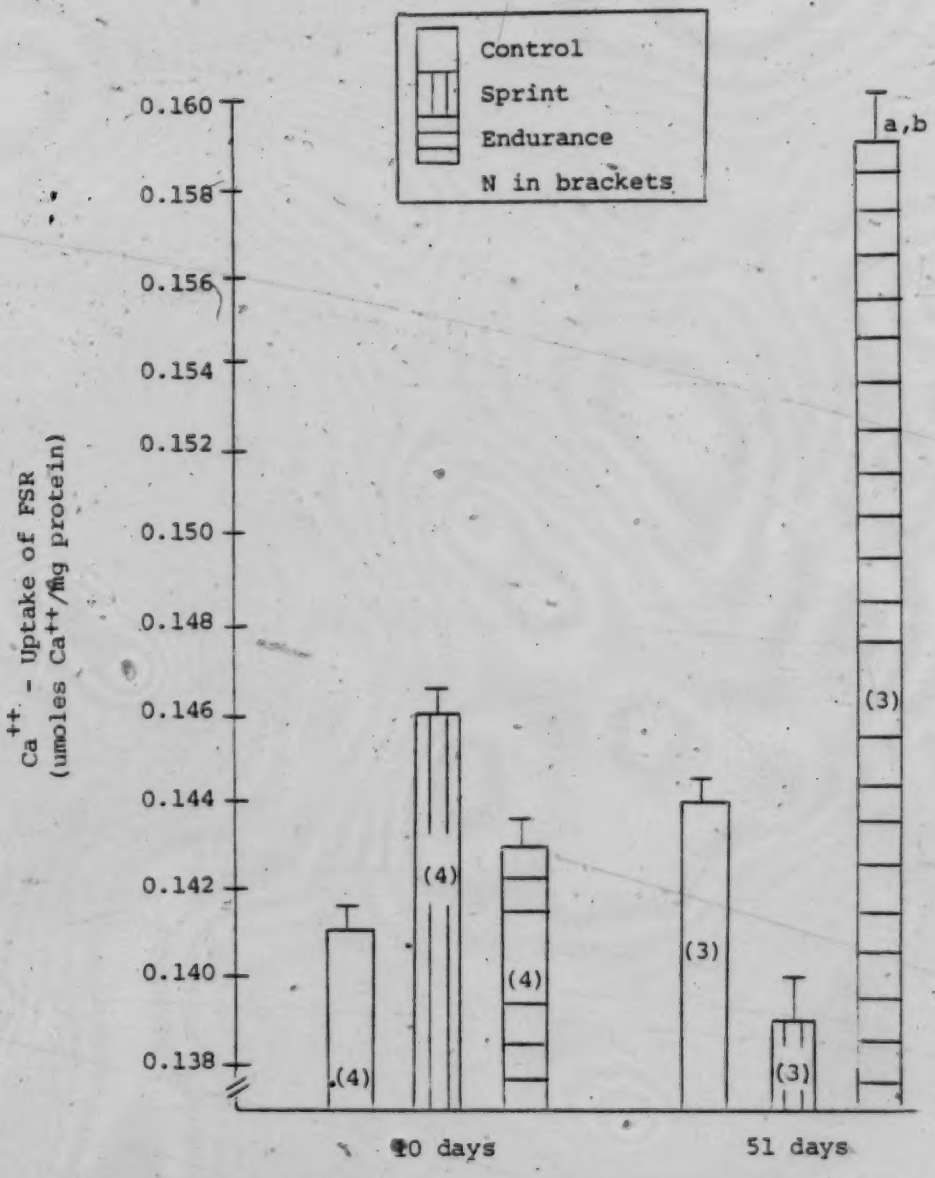

Figure 3:8 Ca^{++} - Uptake of Pooled (2-25 animals) Soleus Muscle ($\bar{X} \pm$ SEM). In order to make up an N of 1 for the 10 day animals, the muscle of 25 animals had to be pooled. For the 51 day animals the muscles of 3 animals were pooled to give the N of 1.

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a 10d vs 51d ($p \leq 0.05$)

b S vs E ($p \leq 0.05$)

5. Fiber Types

The plantaris and soleus muscles of 10 day animals showed a predominately fast-oxidative-glycolytic (FOG) pattern. The soleus had 87% FOG and 13% SO, while the plantaris exhibited 90% FOG, 8% FG and 2% SO fiber types. Percentages of 89 (SO) and 11 (FOG) versus 87 (SO) and 13 (FOG) were observed for 21 and 51 day soleus muscles, respectively. Plantaris muscles had 59% FOG, 33% FG and 8% SO versus 61% FOG, 35% FG and 4% SO for 21 and 51 day animals, respectively.

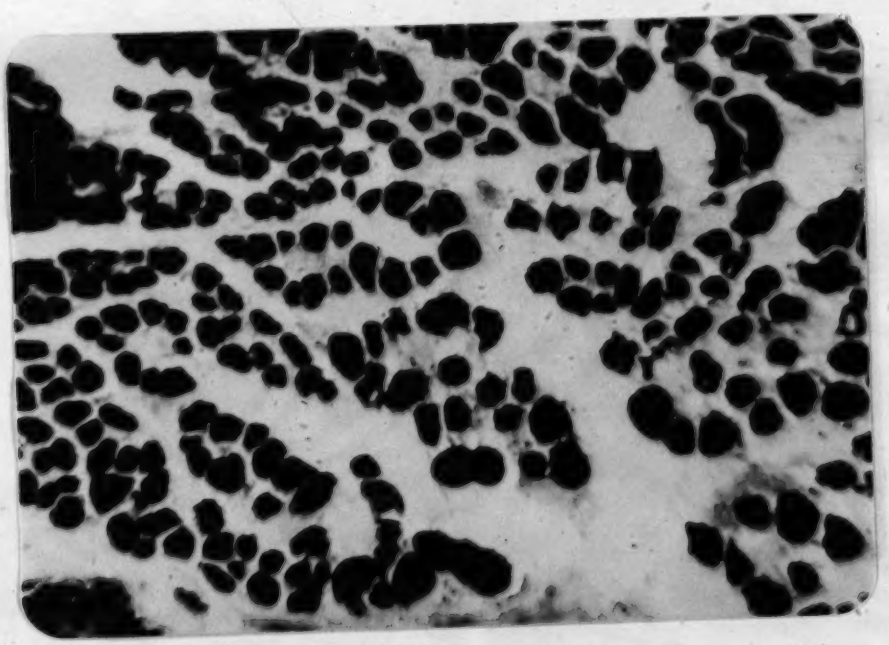
The E animals had more absolute FOG fiber content in both soleus and plantaris when compared to C animals. At 21 days the soleus of E animals were observed to contain 21% FOG and 78% SO fiber types, while the plantaris had 59% FOG, 24% FG and 7% SO fibers. At 51 days, values were 67% FOG, 22% FG and 11% SO fibers for plantaris and 20% FOG and 80% SO for the soleus.

The S animals at 21 days were observed to have 14% FOG and 86% SO for soleus muscles and 62% FOG, 34% FG and 4% SO fibers for plantaris muscle. At 51 days, the plantaris muscle of S animals contained 57% FOG, 38% FG and 5% SO, while the soleus muscle had 18% FOG and 82% SO fibers.

Plate I Micrographs showing serial sections of plantaris and soleus muscles stained for fiber types of control animals.

Plate Ia 10 day Plantaris: Myosin ATPase

Plate Ib 10 day Plantaris: NADH diaphorase



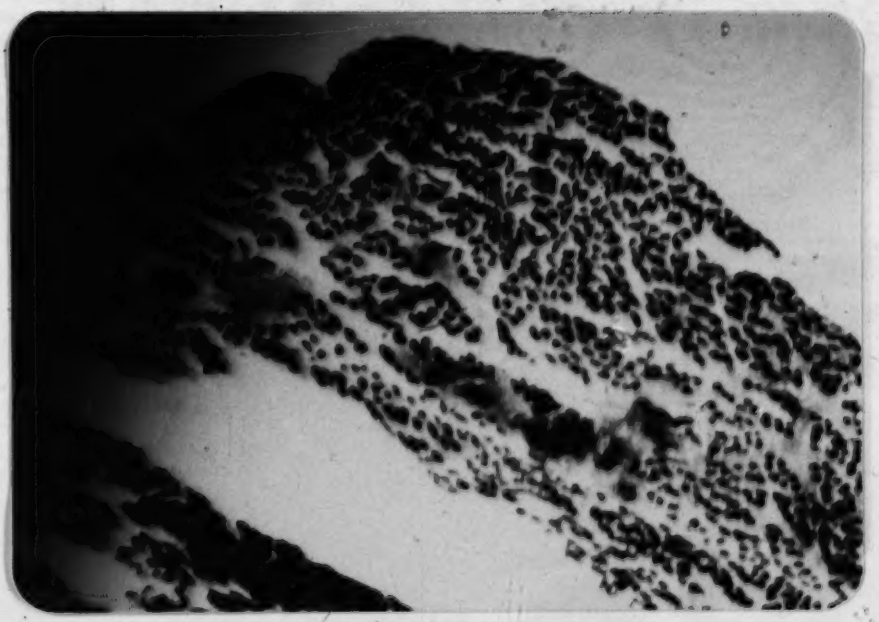
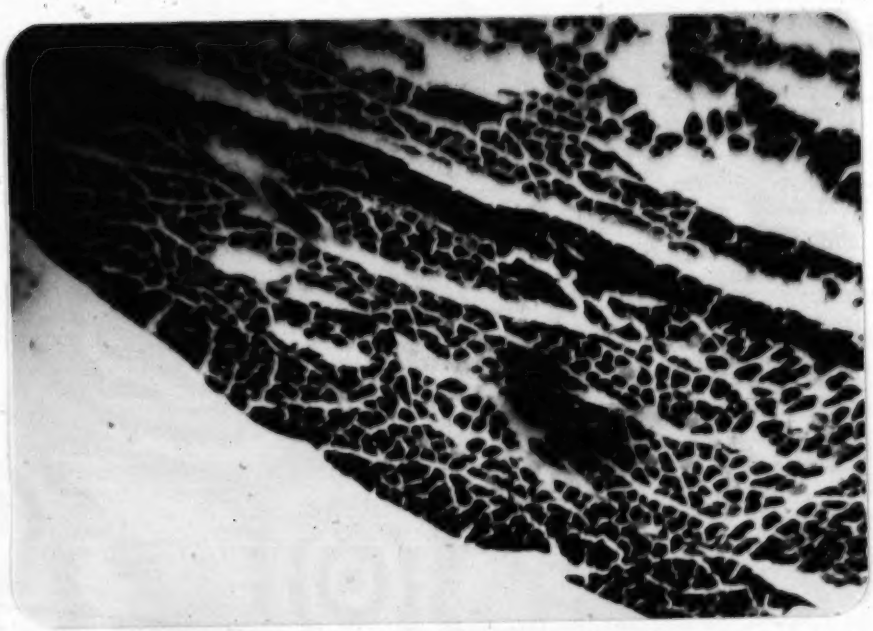


Plate I (continued)

Plate Ic 21 day Plantaris: Myosin ATPase

Plate Id 21 day Plantaris: NADH diaphorase



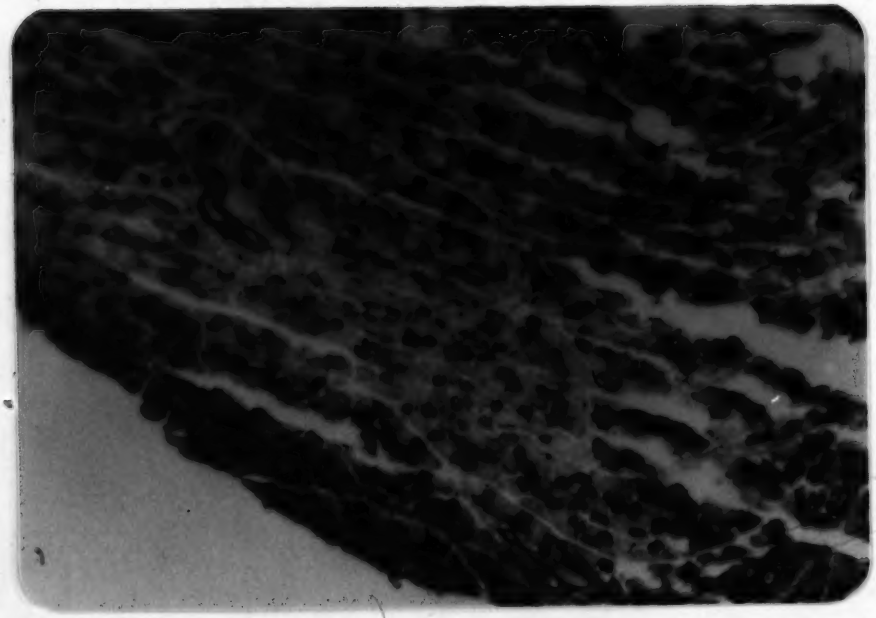


Plate I (continued)

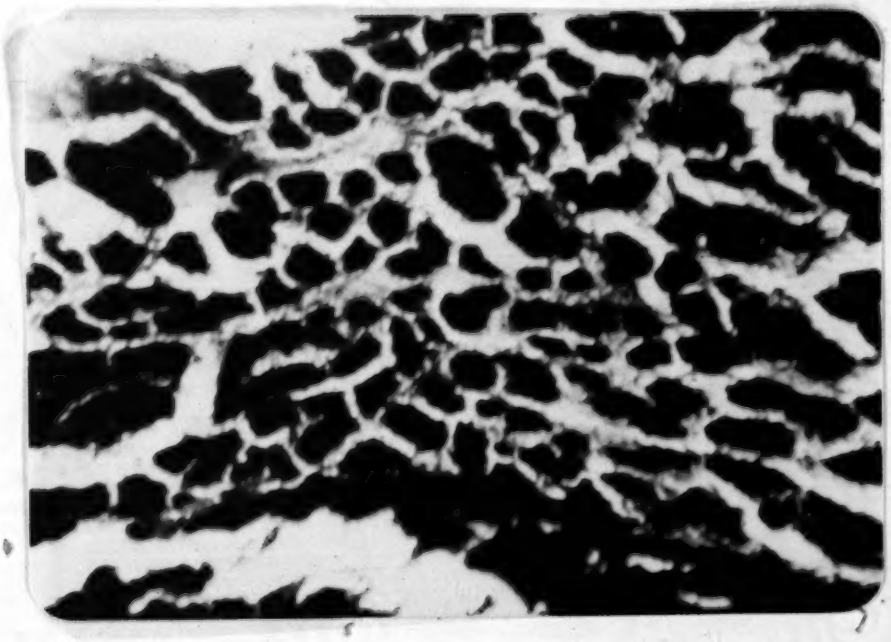
Plate Ie 51 day Plantaris: Myosin ATPase

Plate If 51 day Plantaris: NADH diaphorase

Plate I (continued)

Plate Ig 10 day Soleus: Myosin ATPase

Plate Ih 10 day Soleus: NADH diaphorase



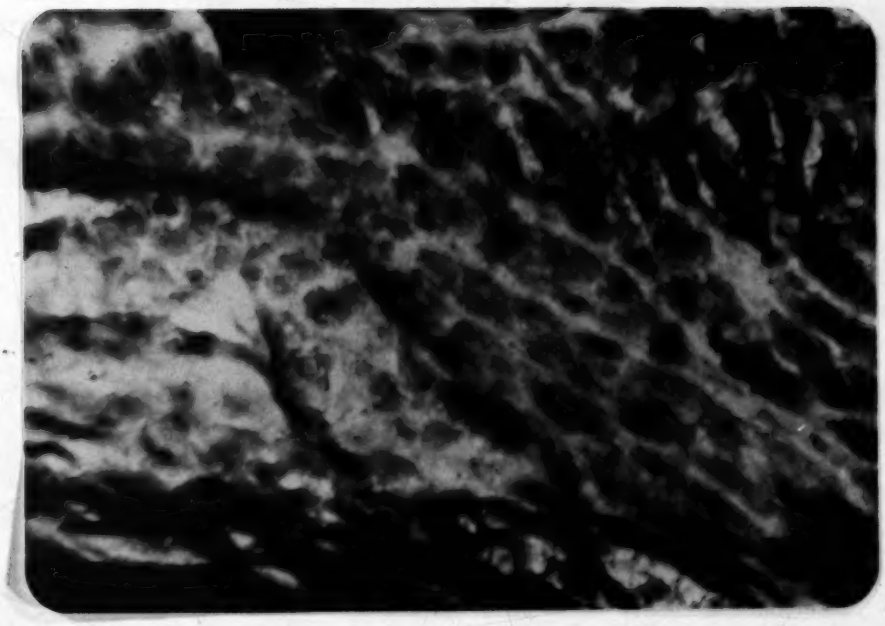
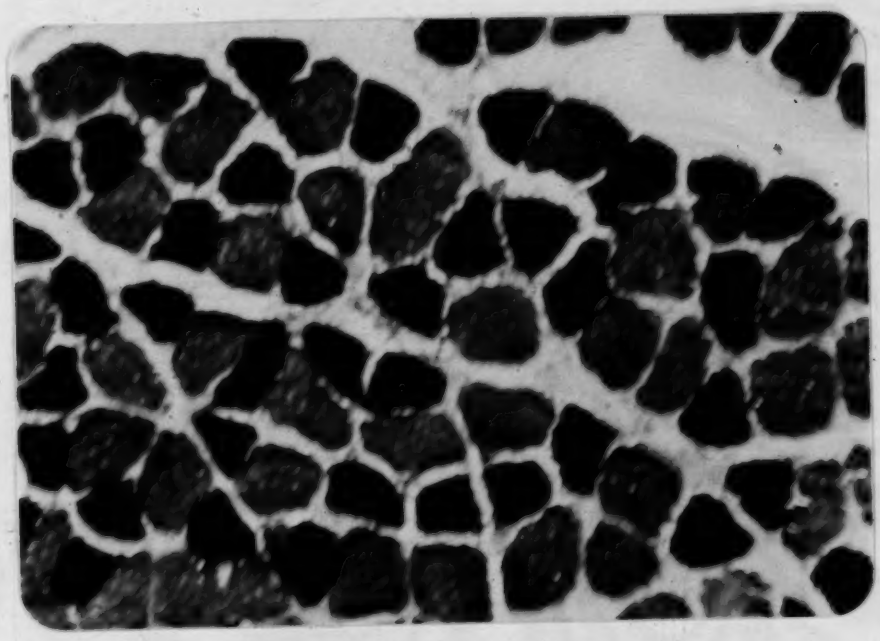


Plate I_6 (continued)

Plate Ii 21 day Soleus: Myosin ATPase

Plate Ij 21 day Soleus: NADH diaphorase



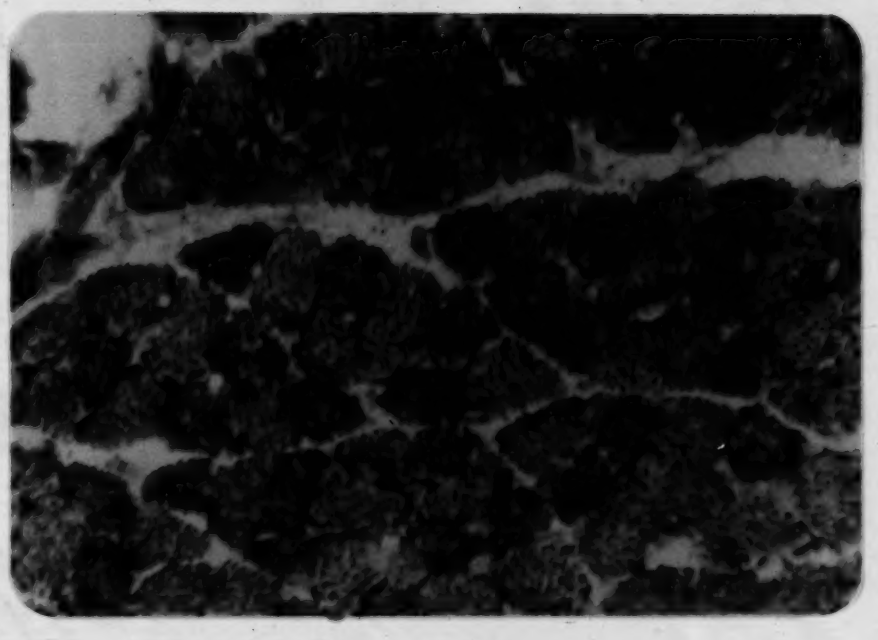


Plate I (continued)

Plate Ik 51 day Soleus: Myosin ATPase

Plate Il 51 day Soleus: NADH diaphorase

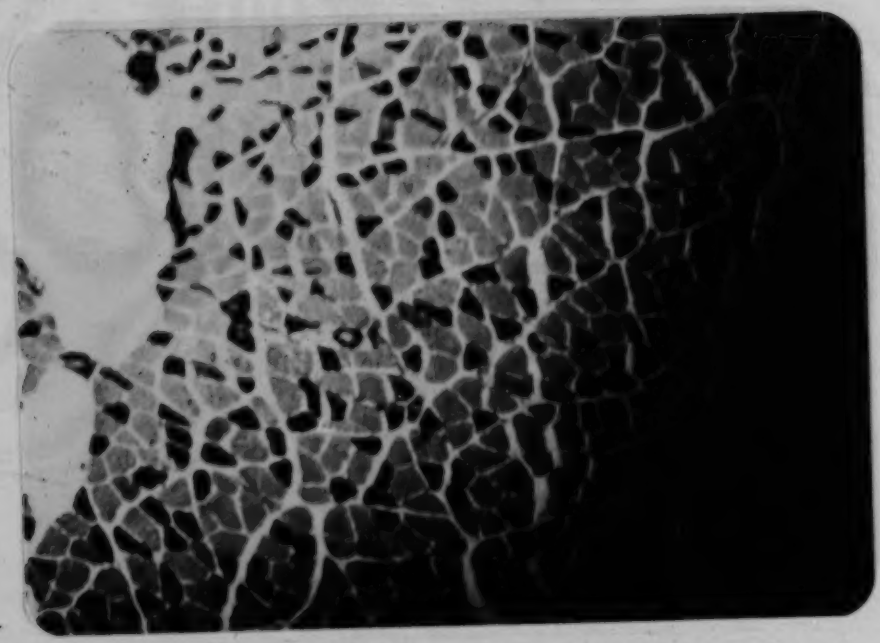


Plate II Micrographs showing serial sections of plantaris and soleus muscles stained for fiber types of endurance animals.

Plate IIa 21 day Plantaris: Myosin ATPase

Plate IIb 21 day Plantaris: NADH diaphorase

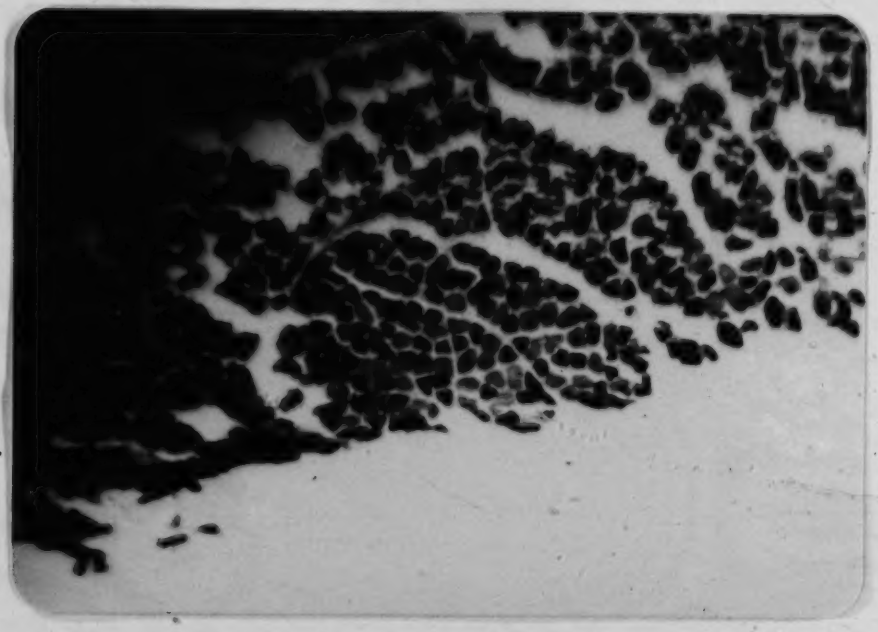
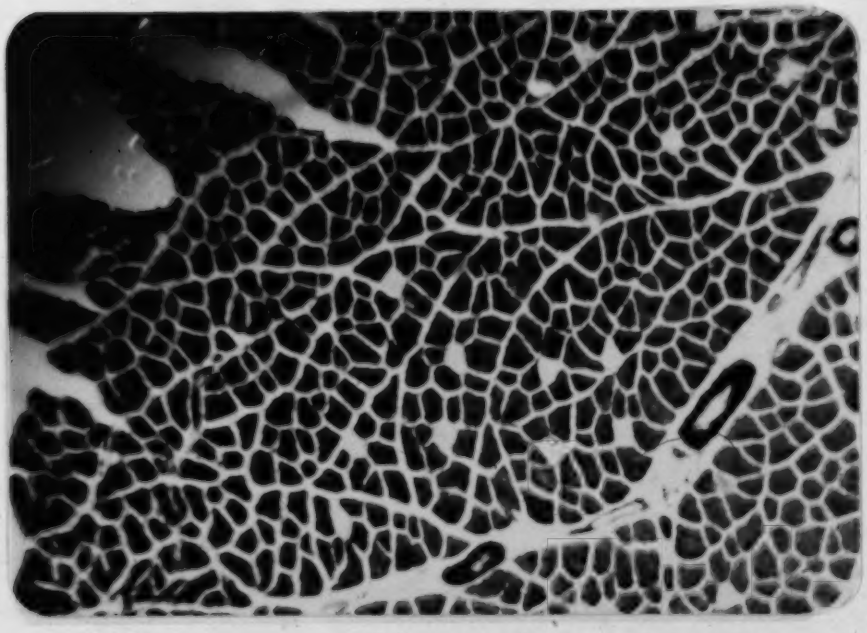


Plate II (continued)

Plate IIc 51 day Plantaris: Myosin ATPase

Plate IId 51 day Plantaris: NADH diaphorase



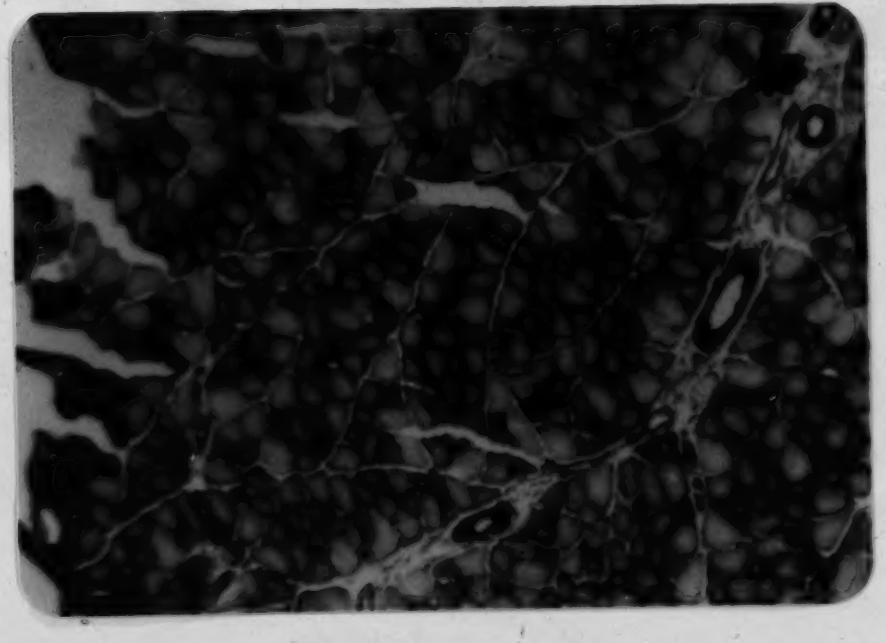
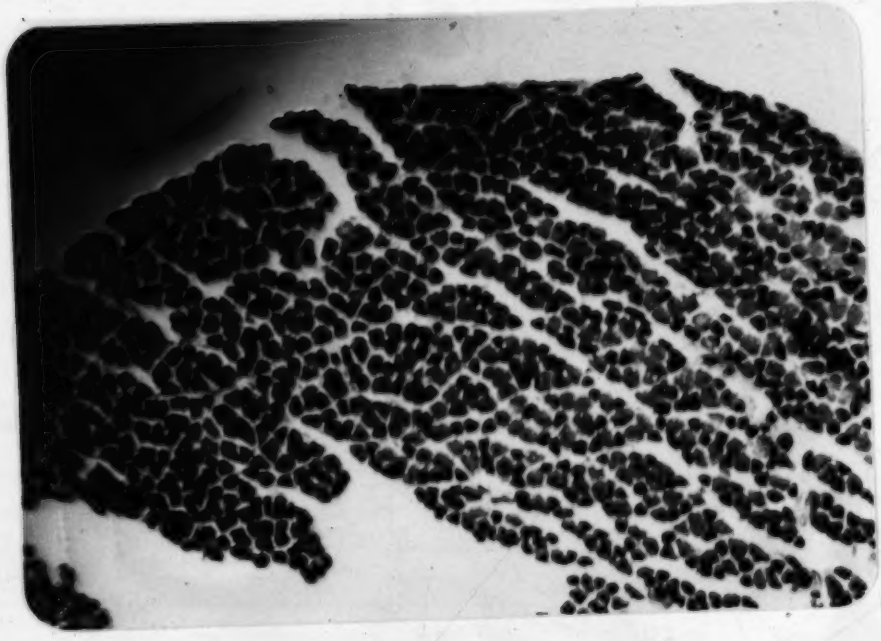


Plate II (continued)

Plate IIe 21 day Soleus: Myosin ATPase

Plate IIf 21 day Soleus: NADH diaphorase



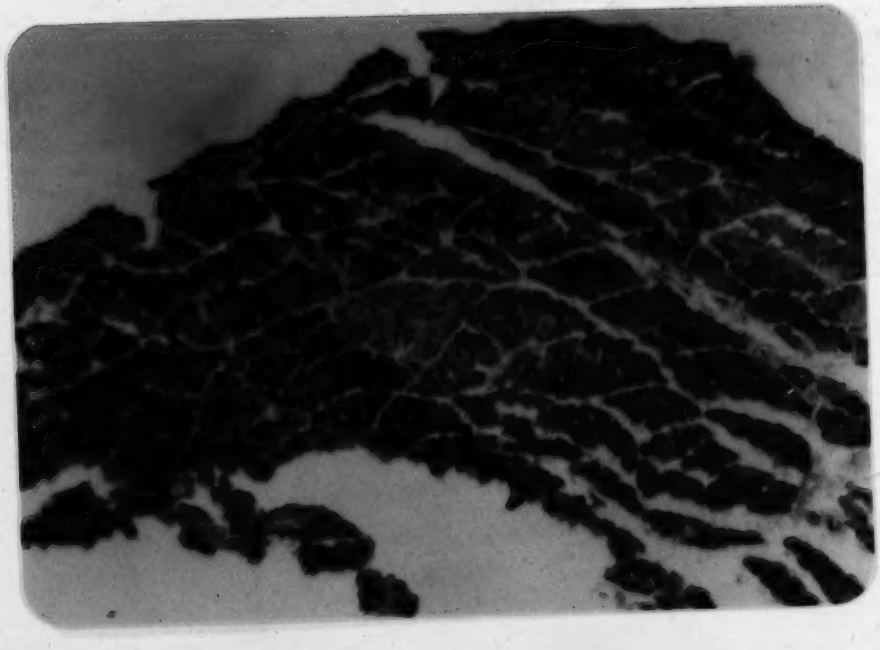


Plate II (continued)

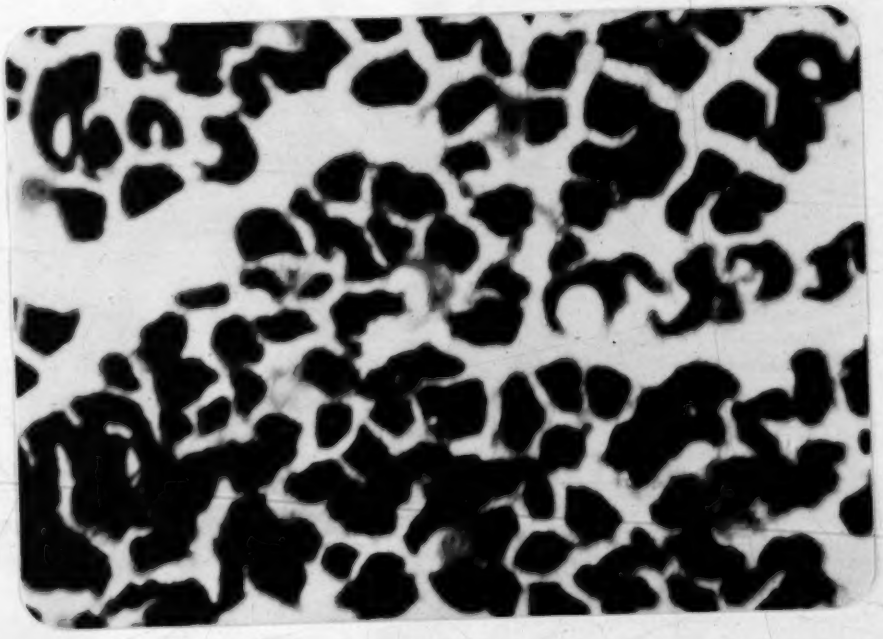
Plate IIg 51 day Soleus: Myosin ATPase

Plate IIh 51 day Soleus: NADH diaphorase

Plate III Micrographs showing serial sections of plantaris and soleus muscles stained for fiber types of sprint animals.

Plate IIIa 21 day Plantaris: Myosin ATPase

Plate IIIb 21 day Plantaris: NADH diaphorase



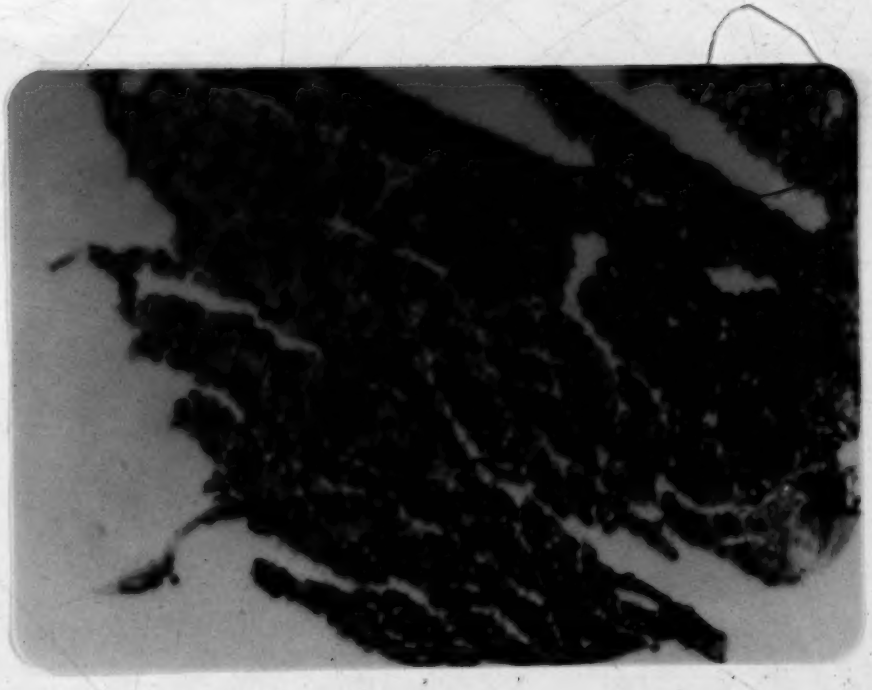


Plate III (continued)

Plate IIIc 51 day Plantaris: Myosin ATPase

Plate IIId 51 day Plantaris: NADH diaphorase

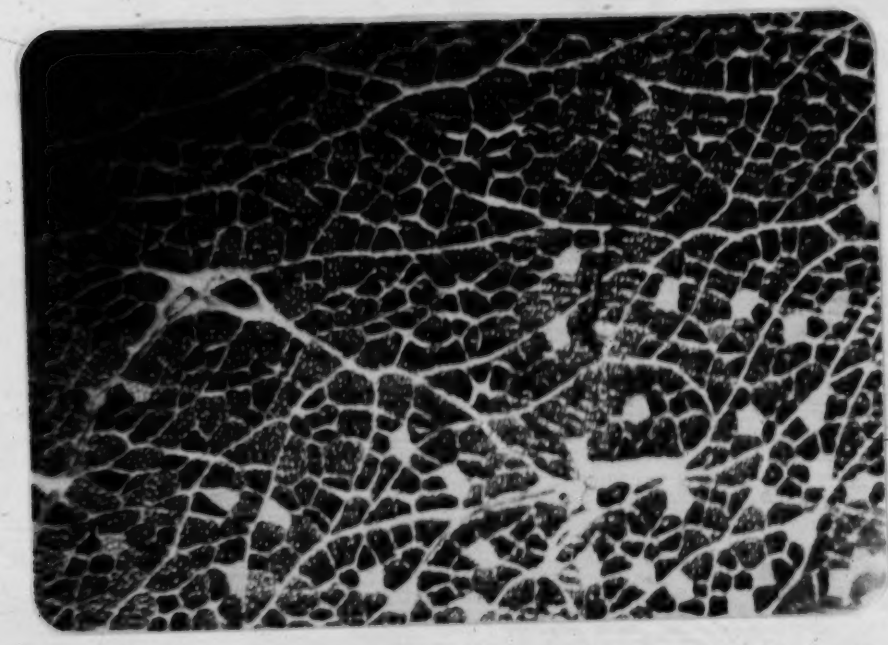
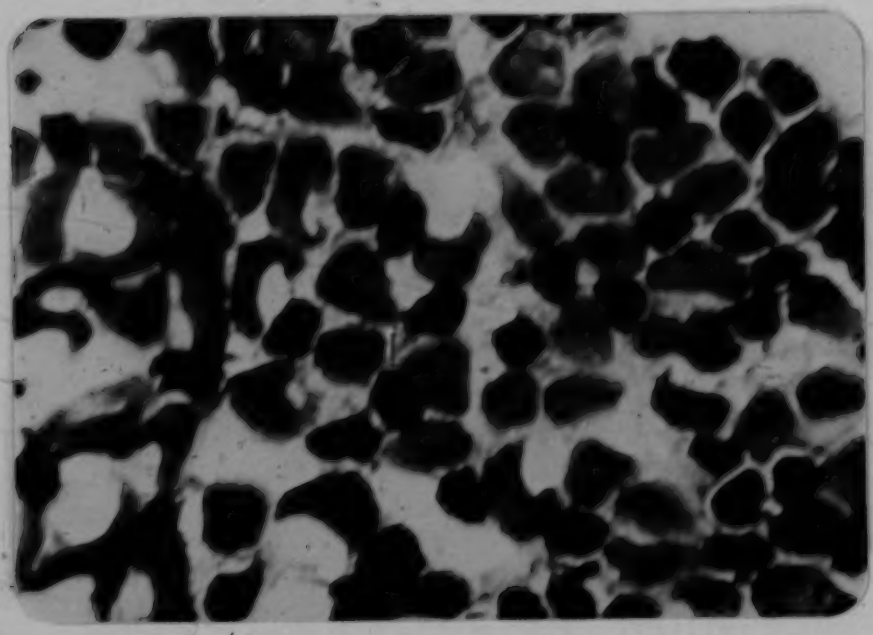


Plate III (continued)

Plate IIIe 21 day Soleus: Myosin ATPase

Plate IIIf 21 day Soleus: NADH diaphorase



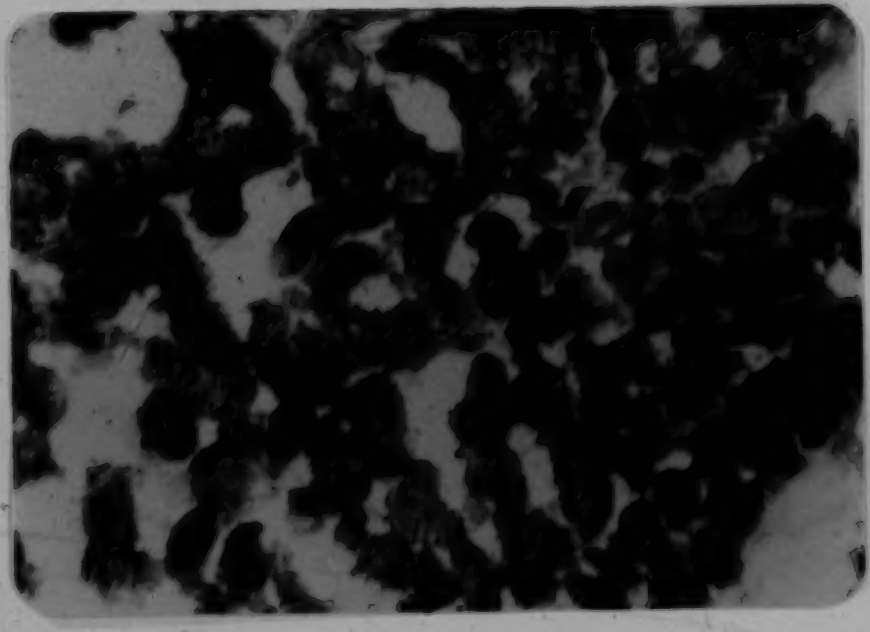
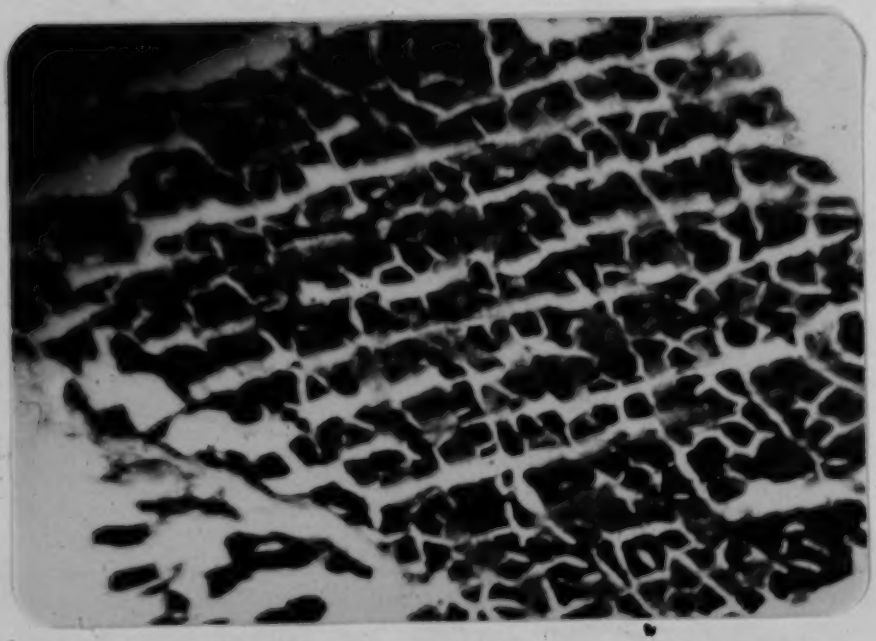
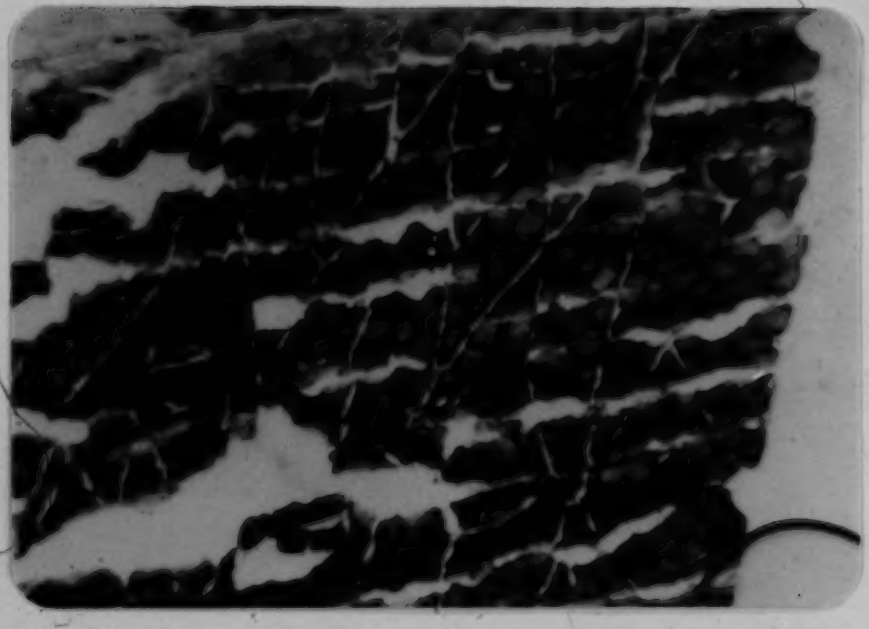


Plate III (continued)

Plate IIIg 51 day Soleus: Myosin ATPase

Plate IIIh 51 day Soleus: NADH diaphorase





DISCUSSION

1. Body and Muscle Weights

The body weight increments observed at each age followed the normal growth pattern typical of maturing animals (Table 3:2) (Gutmann et al., 1974). The lower weights ($p \leq 0.05$) of both the sprint and endurance trained animals are consistent with previous investigations (Houston and Green, 1975; Staudte et al., 1973).

Maturation had resulted in heavier, plantaris and soleus muscles (Table 3:2). The 21 day weights were not effected by the exercise programs ($p \geq 0.05$), which confirms earlier studies employing young, swim trained rats (Gutmann and Hajeck, 1971). At 51 days the plantaris muscles from E and S animals had lower weights ($p \leq 0.05$) than age matched controls, while the soleus muscles weights did not seem affected by either training regimen. Although only 2 muscles were sampled others may have increased, thereby adding to the body weight increments.

The significantly lower plantaris weights at 51 days in the training groups are opposed to previous results with trained animals (3 m/min, 65% grade, 2 hr/day) (Baldwin et al., 1976). The plantaris weight/body weight ratio increases were attributed solely to heavier plantaris muscles. The comparison of plantaris muscle weights between the previous study and this investigation may not be valid, due to the extremely different training protocols and the age of the animals. Also, the lower plantaris muscle weights may not have been a specific function of the training program, since other factors such as littermate

placement and/or sampling technique may have confounded these results. Although the present study attempted to use littermates in each experimental group, this objective was only partially completed due to several factors; number of offspring, deaths and injuries. As well, the origin of the plantaris tendon was difficult to assess which may have been responsible for partial excision of the muscle. This also would tend to artifically lower the plantaris muscle weights reported in this study.

At 51 days, the soleus muscle weights for the C, S and E animals were similar ($P \geq 0.05$) (Table 3:1). Fitts and Holloszy (1977) reported that the length and/or weight of soleus muscle of endurance trained rats were not affected by exercise, when compared to sedentary, control rats.

2. Plantaris Muscle

It appeared that the protein concentration of both total and myofibrillar fractions was relatively uneffected by training, except perhaps at 21 days (Table 3:2). The developmental process in the C group resulted in higher total protein concentrations at each successive age ($p \leq 0.05$). Failure of the training programs (E and S) to alter total protein levels at 51 days, is in agreement with previous reports (Baldwin et al., 1977; Gordon et al., 1967). Similarily, the myofibril or contractile protein fraction of rat skeletal muscle (gastrocnemius) was not statistically changed by training (Wilkerson and Evonuk, 1971).

At 21 days, the plantaris muscle total protein concentrations of E and S animals were higher than the values at 51 days ($p \leq 0.05$). This pattern is different than the total protein concentrations exhibited by muscles of the C group (Table 3:2). Although the total protein concentration of plantaris muscle for the S group at 21 days approximated that of the E group, this value was not statistically different than either the E or C group. The total protein concentration of the muscles from 21 day E animals was greater as a consequence of the training program compared to the C group ($p \leq 0.05$) but no different than the S animals. The results of this investigation suggest that the elevated total protein values were not due to increased myofibrillar protein, since all concentrations, at any age, were not changed ($p \geq 0.05$). Perhaps the greater oxidative potential, which does increase with endurance training (Holloszy et al., 1975), may partially account for the higher total protein levels observed for muscles of E group animals. This explanation seems plausible, especially when the histochemical evidence suggests that a greater proportion of fast-oxidative-glycolytic (FOG) fibers were present in muscles of the endurance trained animals. As well differences in the protein fractions may have been attributed to water shifts occurring in the muscle due to training.

The lowered total protein concentration of muscles from the 51 day E and S animals, when compared to the 21 day values ($p \leq 0.05$), perhaps resulted from a decreased amino acid (^{35}S - methionine and ^{14}C - leucine) incorporation rate that accompanies maturation (Narayana and Eapen, 1975; Gutmann and Syrovy, 1967).

The greatest incorporation rates appear to occur during the first 20-30 days following birth (Narayanan and Eapen, 1975). Thus, the lowered amino acid incorporation rates, as well as the lowered plantaris muscle weights may have partially contributed to the total protein results of this study at 51 days.

The normal developmental pattern of myofibrillar ATPase activity for predominately fast rat muscle was confirmed (figure 3:1). Although previous investigations have employed the extensor digitorum longus (EDL) muscle of rat (Gutmann et al., 1974; Drachman et al., 1973), similar increases in the myofibrillar ATPase activity between 10 and 21 days for plantaris muscle resulted. As well the 51 day C animals had maintained the ATPase activities, which is typical for maturing rat fast muscle types. This pattern is consistent with the contraction times reported for growing rat muscle (Gutmann et al., 1972). Since the contraction time and ATPase activity correlate well with each other ($r^2 > 0.9$) (Drachman et al., 1973; Barany, 1967), it may be suggested the plantaris muscles from C animals had increased the contraction speed from 10 to 21 days.

At 51 days the plantaris muscles of E animals may have shown a prolonged contraction time, particularly since a 23% decrement occurred for Mg^{++} - activated myofibrillar ATPase activity when compared to the 21 day levels ($p \leq 0.05$). This decrease was also evident when the E group was compared to either the 51 day S or C animals (figure 3:1). The lowered ATPase activity for E animals

is in agreement with a report in which a 20% decrease in ATPase activity of rat red vastus muscle (FOG) was attributed to endurance training (Baldwin et al., 1975). It may be that the observed decrease in Mg^{++} - myofibrillar ATPase activity of plantaris from E animals is associated with the FOG proportion of muscle fibers due to the recruitment characteristics. This suggestion is supported by the observation of Burke and Edgerton (1975) that FOG fibers appear to be recruited for many more types of activities than either the fast-glycolytic (FG) or slow oxidative (SO) muscle fibers. As well, the slightly greater FOG fiber population retained in the plantaris of 51 day E animals, compared to the C and S animals, perhaps indicates that myofibrillar ATPase adaptations may also be indirectly supported by the S group. In this investigation the myofibrillar ATPase activity of plantaris muscle of 51 day S animals has slightly greater FG fiber content and it appears that the ATPase activity of this fiber type does not adapt to endurance training (Baldwin et al., 1975).

The "function-fiber type" theory referred to in the introduction, as well as suggested by Baldwin et al. (1975), may be partially demonstrated by these results. The effect of S training, although not altering the myofibrillar ATPase activity as compared to C animals (figure 3:1), appears to have a slightly higher proportion of FG fiber types. As well, the E animals do appear to have a higher proportion of FOG fiber types. The suggestion that the energy supply and demand system are linked

(Baldwin et al., 1975), lends further support to the "function-fiber type" theory. Although the histochemical evidence reported is not conclusive, it does suggest that activity may alter the type of metabolic system preferred by a muscle fiber type. Similarily, the decrement associated with myofibrillar ATPase activities of E animals lends credence to the supposition that function may alter the normal course of development for young rat plantaris muscle. Whether the altered myofibrillar ATPase activities reflect actual molecular re-arrangements of the proteins or simply an attenuation of the proteins already present may not be deduced directly. However, it may be suggested from other reports (Pette et al., 1976; Sreter et al., 1974) that the molecular properties of contractile proteins may exist as an isozyme mixture.

The yield of fragmented sarcoplasmic reticulum (FSR) protein had increased between 10 and 51 days for all groups ($p \leq 0.05$) (figure 3:2). Maturation accounted for increases in ATP-dependent Ca^{++} binding and uptake abilities by the FSR ($p \leq 0.05$) (figures 3:3 and 3:4) which confirms an earlier study (Drachman et al., 1973). It appears that the Ca^{++} related activities necessary for muscular contraction in fast type muscle remains consistent with the contractile properties and proteins throughout development.

Following training (S and E programs) no exercise effect was observed for FSR protein yield and ATP - Ca^{++} uptake ($p \geq 0.05$) in the plantaris. However, at 51 days the muscles from E animals had a greater capacity to bind Ca^{++} than the C group ($p \leq 0.05$).

This enhanced Ca^{++} binding is in opposition to the results of a previous investigation which observed no training (continuous/interval work) changes in the ability to bind Ca^{++} of either guinea-pig gastrocnemius or plantaris muscle (Barnard et al., 1970). This difference may well be a function of the animals species, age of animals and/or intensity and duration of the training programs employed by the investigators. Evidence that the Ca^{++} related activities of skeletal muscle are changeable has been reported under chronic conditions, such as cross-innervation (Mommaerts et al., 1969) and electrical stimulation (Ramirez et al., 1974). The Ca^{++} uptake activity, of cross-innervated cat flexor digitorum muscle by soleus nerve, was reversed after six months, while the reverse experiment required one year before changes had resulted. The FSR electrophoretic pattern of chronic electrically stimulated (8 hr/day, 10 impulses/sec) rabbit tibialis anterior muscle was similar to that of slow muscle. No data on stimulated soleus muscle was reported (Ramirez et al., 1974). Thus, the Ca^{++} related activities of selected skeletal muscles seem to have the ability to adapt to a chronic stress.

The significance of the elevated Ca^{++} binding may be related in part to the fast type of muscle studied (plantaris). It has been suggested that the free Ca^{++} removed from the sarcoplasm by an ATPase - Ca^{++} - pump mechanism is stored with a Ca^{++} binding protein (MW 55,000d), which undergoes configurational changes when bound to Ca^{++} (Gergely, 1974). Moreover, it was postulated that the Ca^{++}, when stored or bound in this form can be much more

easily dissociated and thereby released from the sarcoplasmic reticulum into the actomyosin system. The ATPase - Ca^{++} - pump mechanism which is responsible for Ca^{++} uptake by the sarcoplasmic reticulum has been suggested as one of the distinguishing features between FSR of fast and slow muscles (Fiehn and Peter, 1971). This may in part be related to the capability of the FSR of fast muscle to bind more Ca^{++}, since the ATPase - Ca^{++} - pump mechanism has been reported to be inhibited by large concentrations (0.1 mM) of free Ca^{++} (Gergely, 1974). Furthermore, Sembrowich and Gollnick (1977) suggested that the Ca^{++} uptake of skeletal muscle (gastrocnemius) is lowered as a function of exercise intensity. Thus, the enhanced Ca^{++} binding ability of plantaris muscle may reduce the free Ca^{++} and hence lower the Ca^{++} levels to those which are optimal for the ATPase - Ca^{++} - pump. This should then enhance the recovery phase of contraction. These changes may be of physiological significance in the exercising muscle which must not only prepare the internal cellular environment for relaxation, but also be prepared to release Ca^{++} so that the contractile proteins have the required Ca^{++} necessary for contraction.

3. Soleus Muscle

The training (S and E programs) employed in this study from 10 to 51 days of age had no observable effect upon total protein concentrations at any age (Table 3:3). As well, the developmental process did not appear to alter the total protein concentration

between 10, 21 and 51 days ($p \geq 0.05$). These results may obscure the possibility that different rates of protein synthesis may have been occurring in various subcellular fractions yet, the total protein content remained relatively stable. The different incorporation rates of amino acids (^{35}S - methionine) into either the myofibrillar or sarcoplasmic reticulum fraction with increasing age (Narayanan and Eapen, 1975) may lend support to the possibility of total protein not being composed of equal proportions of subcellular fractions during development. The myofibrillar protein fractions of soleus muscles from 21 day S and E animals are higher ($p \leq 0.05$) than the C values (Table 3:3). As well, from 10 to 51 days the FSR protein yield is decreasing (figure 3:6). Thus, the net result, excluding other possible protein fractions, suggests a relatively stable total protein concentration.

The pattern of soleus myofibrillar ATPase activity exhibited in this report (figure 3:5) by the C animals closely resembles the normal maturation phenomena (Gutmann et al., 1974). At 51 days the muscles of E and S trained animals had greater ATPase activities than C animals ($p \leq 0.05$) which confirms earlier reports (Baldwin et al., 1975; Syrovy et al., 1972). Baldwin et al. (1975) have suggested that ATPase adaptations may be brought about by either complete transformation of the myosin molecule or by partial replacement of the two different forms of myosin, resulting in a mixture of both enzyme types. Whether the changes reported in this study or by Baldwin et al. (1975) are changes unique to Ca^{++} - Mg ATPase or to Mg^{++} ATPase or some combination of

both was not ascertained. Syrovy and Gutmann (1977) have observed that young soleus myosin is associated with LC_{1s}, LC_{1f}, LC_{2s}, LC_{2f} and LC_3 and normal adult muscle has only LC_{1s} and LC_{2s}. If the myosin light chain component of the soleus muscle followed a normal maturational pattern in C animals, the myofibrillar ATPase alterations for muscles of 21 and 51 day S and E animals may be related to the enhanced neural activity delivered to the soleus during training. Since the myofibrillar ATPase has been shown to be a function of the light chain properties, the light chains (LC) may have altered as well as the myofibrillar ATPase. The precise function of these light chains is unclear, but two theories have been suggested in a review article by Mannhertz and Goody (1974). The authors suggest that the LC may either serve to stabilize the globular head region, thus allowing ATP to bind and be hydrolyzed or they may in some way aid in direct binding of the ATP molecule to the globular head region. The alteration of myosin lgiht chains due to various forms of "neural" activity, such as electrical stimulation and cross-innervation, also lends support to the contention that soleus myosin light chains may be partially changed. Sréter et al. (1974) have reported that cross-innervation of rat soleus, leads to a partial transformation of the myosin molecule, with a LC_{1f} component clearly identified, as well as a faint band corresponding to the LC_3 component. Partial transformation occurred for the fast EDL muscle, with exhibited a LC_{1s} band, with very faint LC_{2f} and LC_3 proteins after cross-innervation. The loss of LC_3 protein for rabbit tibialis anterior and EDL muscles had resulted following prolonged periods

of both continous and intermittent, electrical stimulation (Pette et al., 1976).

Histochemically, the results suggest that the trained soleus muscle may have a greater potential to participate in a variety of activities, since the high proportion of FOG fiber types present at 10 days is maintained somewhat by the training program. Whether this is the situation in animals that begin training later in their life cycle is unknown.

The ATP-dependent Ca^{++} binding ability and FSR protein yield of soleus muscle decreased with maturation in C animals ($p \leq 0.05$) (figures 3:6 and 3:7). However, the Ca^{++} uptake activity remained relatively constant throughout development (figure 3:8). Drachman et al. (1973) observed that Ca^{++} related activities in the FSR parallel the changes in myofibrillar ATPase activity of developing rat fast muscle. This phenomenon appears to occur in soleus muscle since the FSR yield of Ca^{++} binding (figures 3:6 and 3:7) decreased with the myofibrillar ATPase activity (figure 3:5) during maturation. The failure of Ca^{++} uptake of FSR fractions to parallel the myofibrillar ATPase activity as a consequence of development may be related to the mitochondrial Ca^{++} uptake. The Ca^{++} reduction from the sarcoplasm via the mitochondria occurs readily (Lehninger, 1974). This may have contributed to the unchanged FSR Ca^{++} uptake. Thus, as the muscle matures perhaps greater Ca^{++} uptake by mitochondria, as well as increased number and size of mitochondria, allows for more Ca^{++} to be taken up by this fraction. This suggestion is supported by the

observation that the sarcoplasmic reticulum of slow muscle is not as structured as the fraction obtained from fast muscle (Fabiato and Fabiato, 1977). The time sequence of FSR fraction development in relation to the contractile properties (Pette et al., 1976; Ramirez et al., 1974; Drachman et al., 1973) can not be inferred from the results of the present investigation. However, it may be suggested that the soleus muscle had the potential initially, in terms of FSR related activities, to develop and perhaps retain fast contractile properties. This ability seems to be lost with normal development and may partially be attributed to the muscles anti-gravity function.

The increased FSR Ca^{++} uptake by soleus muscle from E animals ($p \leq 0.05$) (figure 3:9) is in agreement with a previous report for trained (26 week) rats (Bonner et al., 1977). The Ca^{++} activity of the microsomal fraction of soleus muscle increased 28%, while the mitochondrial fraction had decreased 18% following training. Lehninger (1974) suggested that the mitochondrial uptake of Ca^{++} takes precedence over energy production. The evidence by Bonner et al. (1977), that there may be less of a disturbance upon oxidative phosphorylation by mitochondrial Ca^{++} activity, may explain the increased Ca^{++} uptake shown in this study. Thus, following a training program it may be that the FSR fraction has assumed greater responsibility for reducing sarcoplasmic Ca^{++}, thereby allowing the mitochondria to participate to a greater extent in ATP production. The greater involvement of the FSR fraction of soleus

muscle from E animals may be plausible, especially considering that the protein yield for FSR was not reduced as it was with both the S and C groups.

The failure of Ca^{++} binding in soleus muscle to alter with training (S and E animals) may be explained by the FSR releasing mechanism for Ca^{++}. It was postulated that Ca^{++} release is not totally dependent upon depolarization events, as with fast muscle, but rather Ca^{++} movement across the membrane of less structured SR allows Ca^{++} to be made available to the actomyosin complex (Fabiato and Fabiato, 1977). Thus the requirements of Ca^{++} binding to specialized proteins may not be as necessary a feature of slow muscle as with fast muscle.

4. Summary and Conclusions

Two different training programs were used in this study. An intense 6 week sprint and endurance exercise protocol was used to interrupt the normal, developmental pattern of 10 day rat soleus and plantaris muscles. Since the training programs place different metabolic and contractile demands on skeletal muscle and if function does dictate muscle characteristics, then the 2 programs should selectively alter muscle properties. In an attempt to observe exercise changes on growing, rat skeletal muscle, this study used Mg^{++} - activated myofibrillar ATPase activity, Ca^{++} binding and uptake of FSR protein.

Normal developmental patterns of myofibrillar ATPase activities occurred in the plantaris muscle. As well, the enhanced FSR protein yield, Ca^{++} binding and uptake due to maturation suggest that the contractile and SR fractions of growing muscle change in a similar fashion throughout development.

The S training did not effectively alter any of the variables observed. This may be partially related to the inability of the S program employed to substantially overload FG fibers or that the contraction-relaxation characteristics of FG fibers may be sufficiently developed and therefore the sprinting is not perceived as a stressful condition. As well, the selection of animals (e.g. rat) may have contributed to the findings reported.

The E program resulted in lower myofibrillar ATPase activity and greater Ca^{++} binding ability in plantaris muscle. The

The alterations which occurred due to the E program may be explained by the observation that a slightly greater proportion of FOG fibers were retained. This retention since the plantaris is composed primarily of FG and FOG motor units should have resulted in increased recruitment of FOG fibers.

Maturation of soleus muscle resulted in a decrease of myofibrillar ATPase activity, FSR protein yield and Ca^{++} binding ability for the C animals.

The S and E animals both retained slightly greater FOG fibers, at 21 and 51 days when contrasted to the C animals. Both programs had the soleus myofibrillar ATPase activities at approximately similar values as those reported for the 10 day animals.

The enhanced oxidative capacity of E animals may have attributed to the increased Ca^{++} uptake of soleus muscle at 51 days. The evidence that FSR protein yield remained elevated during development supports the suggestion that the FSR fraction appears to participate more readily in the reduction of free, sarcoplasm Ca^{++}. This may allow the mitochondria to generate more energy for the requirements of muscle contraction and not disrupt oxidative phosphorylation as in normal, soleus muscle. The failure of S animals to alter Ca^{++} related activities may be partially accounted for by the inability of the S program to effectively alter the mitochondria of soleus muscle. Thus, the soleus muscle appears to

be influenced by function, especially E exercise, during development by retaining contractile and sarcoplasmic reticulum capabilities that otherwise would have been lost during normal development.

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APPENDIX A

DIFFERENCES AMONG MAMMALIAN SKELETAL MUSCLE FIBERS

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Parameter	Fast-Glycolytic	Slow-Oxidative	Fast-Oxidative Glycolytic
Myoglobin	low	high	high
Glycolytic enzymes	high	low	intermediate
Stored glycogen	high	low	intermediate
Glycogen resynthesis	slow	fast	intermediate
Phosphorylase	high	low	intermediate
Oxidative (Kreb's) Enzymes	low	high	high
Lipolytic enzymes	low	high	high
Stored fat	low	high	high
Myosin ATPase reaction velocity	fast	slow	fast
Lactate uptake	low	high	high
Lactate projection	high	low	low

(con't.)

Parameter	Fast-Glycolytic	Slow-Oxidative	Fast-Oxidative Glycolytic
Location in muscle	usually peripheral	deep core	scattered
Contractile protein	high	low	intermediate
Diametre	large	intermediate	small
Motor-End Plate	large, complex	large, complex	?
Conduction velocity (nerve)	fast	slow	fast
Number of mitochondria	few	many	many
Ca^{++} uptake and release	fast	slow	fast
Type of contraction	phasic	tonic	phasic
Isometric contraction time	fast	slow	fast
Relaxation time	fast	slow	fast
Capillary/fiber ratio	low	high	high
Resting membrane potential (mV)	-85.3	-69.7	-71.7

APPENDIX B

REVIEW OF LITERATURE

REVIEW OF LITERATURE

1. Structure and Function of Myosin

The contractile apparatus which is responsible for the transfer of chemical energy into mechanical work is made up of a number of components. The primary area responsible for the transfer, has been associated with the thick filaments of the contractile apparatus as reported by Huxley (1955), more precisely termed the myosin filament.

The molecular structure of myosin has been well defined in recent years particularly due to advances in analytical ultracentrifugation and proteolytic enzyme digestion. Although the myosin isolated from a number of species has been reported to be different (Brahms and Kay, 1963), general features have been identified. This protein is associated with approximately 5,000 amino acid residues, combined into two identical polypeptide chains in a coiled coil fashion, 1400 Å in length with both hydrogen and hydrophobic bonding (Gazith et al., 1970; Gershman et al., 1969; Lowry et al., 1969). The mass of the protein is large (Mw 500,000d) and various subunits have been identified. The rod shaped myosin molecule has been shown to consist of a light meromyosin (LMM) and heavy meromyosin (HMM) component, with the latter fraction forming two globular head regions. The LMM component comprises approximately 48% while the HMM component makes up 52% of the total myosin (A.G. Szent-Gyorgyi, 1953 a). The secondary structure of the LMM

and HMM has also been elucidated (Kominz et al., 1965; Mueller and Perry, 1962). The LMM has 100% α-helical conformation and retains its solubility characteristics, but no enzymatic activity when separated. The HMM is associated with 50% α-helix form, is water soluble and retains the enzymatic activity characteristic of myosin. With further digestion by large concentrations of trypsin, the HMM fragment may be divided into HMM-subfragment 1, and HMM-subfragment 2, with only the HMM-S_1 subunit retaining the enzymatic activity and actin-binding characteristics, (Lowry et al., 1969; Mueller and Perry, 1962). Associated with the HMM-S_1 fraction are low molecular weight components or light chains (L.C.), which are not covalently linked to the globular head region, (Mannhertz and Goody, 1974: Weeds, 1969).

The precise function of these L.C. are unclear, but two theories have been suggested in a review article by Mannhertz and Goody (1974). Briefly, they suggest the L.C. may either serve to stabilize the globular head region, thus allowing ATP to bind and be hydrolyzed or they may in some way aid in direct binding of the ATP to the globular head region. In any event, the importance of the L.C. is made clear upon separating them out, which results in a loss of ATPase activity.

The exact numbers and masses of the various light chains are different between FG and SO muscle fibers (Weeds and Pope, 1971). FG skeletal fiber contains three different L.C. on each of the two globular head regions. Two of the light chains have been separated

out at a pH of 11, and thus termed the alkaline chains of LC_1 and LC_3, with masses of 23,000d and 16,000d, respectively, (Sarkar et al., 1971; Gershman et al., 1969; Frederiksen and Holtzer, 1968). The remaining light chain (LC_2), also termed the DTNB[1] chain with a molecular weight of approximately 18,000d does not appear to be functionally related to the ATPase activity of myosin, since the ATPase activity is still present when LC_2 is extracted. With regard to SO muscle fibers, only two different LC were found by electrophoretic analysis, with molecular masses of approximately 26,000d and 17,000d, (Weber and Oplatka, 1974; Sreter et al., 1972).

2. Neural Regulation of Contractile Characteristics

The differences observed between various muscle fiber types have been attributed to the neural supply associated with the muscle (Vrbova, 1963; Bach, 1948). The reliance of muscle fiber characteristics and properties upon neural input was substantiated by atrophy resulting from denervation. Bajausz (1964) found that white fibers have a relatively greater dependence on a nerve-muscle interaction than red fibers in regulating fiber size. Also Melichna and Gutmann (1974) have suggested that neural activity is important in regulating contractile properties by denervation and immobilization studies. Buller, Eccles and Eccles

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[1]Because of extraction with 5,5'-dithio-bis (2-nitrobenzoic acid).

(1960 a,b) also attributed the nerve-muscle interaction as regulating the contractile properties characteristic of individual fiber types by spinal cord section and cross-innervation studies. Luff (1975) found that in cat soleus (SO) and EDL (FOG) muscle, the time to peak tension and intrinsic speed of shortening was reserved after cross-innervation. This particular phenomenon has been further substantiated by a number of investigators with a summary of their results appearing in table one. The precise mechanism responsible for these changes is uncertain. For example, whether or not the nerve acts directly or by some trophic substance is unclear, but the nerve-muscle interaction related to contractile characteristics is no longer in doubt.

Changes in contractile properties by cross-innervation, have lead several investigators to study the contractile proteins responsible for contraction. Since it is known that a difference in contractile proteins exist between FG and SO fiber types, it was expected that these should also be altered after cross-innervation. The proportional relationship between contractile properties (e.g. speed of shortening) and myosin ATPase activity (Barany, 1967) lead to the idea that the myosin molecule was the important feature of the contractile apparatus that was subject to change. Hoh (1975) suggested that myosin is probably the site where neural activity affects the contractile properties (e.g. speed of contraction).

Buller et al. (1969) found that in both cat and rat muscle, which had been transformed in terms of their contractile

TABLE ONE

Review of Some Cross-Innervation Studies

Researchers	Species	Type of Contraction	Parametre	Results* (msec)	
Buller et al. (1960 b)	Cat	Twitch	Contraction Time	N - Sol. 60 X - Sol. 42	N - FDL 33 X - FDL 59
Buller and Lewis (1965)	Cat	Twitch	Absolute refractory period	N - Sol. 1.6 X - Sol. 1.1	N - EDL 1.0 X - EDL 1.1
Close (1969)	Rat	Twitch	Contraction Time	N - Sol. 34 X - Sol. 15	N - EDL 13 X - EDL 25
Buller et al. (1969)	Cat	Twitch	Time to Peak Tension	N - Sol. 75 X - Sol. 35	N - FDL 25 X - FDL 59
Barany and Close (1971)	Rat	Twitch	Contraction Time	N - Sol. 37.8 S - Sol. 38.0 X - Sol. 15.6	N - EDL 12.56 S - EDL 12.54 X - EDL 21.33

(con't.)

Researchers	Species	Type of Contraction	Parametre	Results* (msec)			
Sreter et al. (1974)	Rat	Twitch	Time to Peak Tension	N - Sol. 37.78 X - Sol. 16.19		N - EDL 14.20 X - EDL 24.84	
Luff (1975)	Cat	Twitch	Time to Peak Tension	N - Sol. 84.7 X - Sol. 33.9		N - EDL 23.4 X - EDL 52.6	
Luff (1975)	Rat	Twitch	Time to Peak Tension	N - Sol. 39.5 X - Sol. 19.7		FDL ? FDL ?	

* N = normal
X = cross-innervated
S = self-reinnervated

Sol. = soleus
EDL = extensor digitorum longus
FDL = flexor digitorum longus

properties due to nerve alterations, the Ca^{++} activated ATPase activity was altered. Barany and Close (1971) found that changes in the contractile properties reflected the changes at the molecular level (i.e. ATPase activity). Alterations in myosin ATPase changes paralleled the transformation of FG muscle to SO muscle. Decreases in myosin ATPase activity as FG muscle was transformed into SO, suggested the importance of molecular changes in determining the characteristics of different fiber types. (Baldwin et al., 1976; Samaha et al., 1970).

The myosin molecule of FG and SO fiber types are associated with different light chain components. Thus, the effect of cross-innervation have also been examined in terms of light chain transformations. The adaptation of the myosin molecule occurred through changes in the number of light chain components (Pette et al., 1976; Hoh, 1975; Gutmann et al., 1974; Sreter et al., 1974) and amino acid sequences (Huszar, 1972). Thus, it appears that the neural activity associated with a muscle is important in regulating its contractile properties, which reflect structural changes at the molecular level.

3. Developmental Consideration

The differentiation of FG and SO fibers after birth may be demonstrated by differences in contractile properties (Close, 1964). The reliance of the neural supply to muscle differentiation was discussed previously, but the importance of neural activity during

development must also be considered. Brown (1973) demonstrated that the normal pattern of time to peak contraction was levelling off near adult values at approximately 3 weeks of age for rabbit EDL (FOG) and soleus (SO) muscles, but if denervation of EDL occurred, the muscle did not develop these fast twitch characteristics. On the other hand denervated, soleus muscle continued its normal developmental pattern. When the EDL was electrically stimulated (i.e. trains of 500 msec, every 2 min. for duration of 0.5 msec at frequency of 40 sec -1), after denervation its contractile properties were once again restored. A similar pattern of development has been found for rat muscle, except that the time to reach contractile maturity occurs approximately 4 weeks after birth (Gutmann et al., 1973; Gutmann and Melichna, 1972).

Immediate post-natal changes in the rate of protein synthesis in SO and FG chicken muscle, again points to the importance of the post-natal developmental period with regard to muscle differentiation. Narayanan and Eapen (1975) found that greater protein incorporation occured in the myofibrillar region as compared to the sarcoplasmic regions of FG and SO muscle, 20 and 30 days respectively, after hatching. Thus, structural changes occuring at this early stage of development may well influence the functional properties expressed by a particular muscle fiber type, during its adult life.

Calcium-activated myosin ATPase activity for developing rats is lower than in mature rats due to possible changes in the

kinetic properties and active site conformational changes expressed by light chain components and sulfhydryl group content. For rabbit gastrocnemius and soleus muscles the percentage of HMM increased by 27% and 18% from birth to 25 days of life, after which normal adult levels had been reached and were stabilized (Pelloni-Mueller et al., 1976). In addition, for rat muscle, it was found that approximately 21 days after birth, the EDL (FG-FOG) muscle had reached contraction times resembling those of adult muscle, with a correlation of .91 between speed of contraction and myosin ATPase activity (Drachman and Johnston, 1973). The myosin ATPase activities paralleled the developmental changes of contraction time for both rat EDL and soleus muscles (Gutmann et al., 1974). These investigators found a decrease in contraction of time of EDL and soleus between one week (25 and 47 msec) and one month (11 and 26 msec) old rats, while after this period, contraction times increased until 12 months of age (15 and 38 msec). The myosin ATPase activities, between 1 and 4 months of age, for EDL muscle increased (.91 to .98 ugPi/mg/min), while the activity for soleus decreased (.51 to .45 ugPi/mg/min). The investigators demonstrated that physiological changes during early stages of development, are associated with biochemical alterations, similar to reports on adult animals. Also, light chain components followed a similar pattern, and thus the authors have suggested a loss or transformation of the fast type of myosin light chains, associated with the development of soleus muscle.

The kinetic properties of myosin ATPase changed from 1 to 24 months of age for rat muscle. Kaldor and Mim (1975) have suggested that during development there could be an alteration of or near the active site or sites of the myosin molecule, due to the following observations; 1) the maximum velocity (V_{MAX}) of the ATPase reaction was significantly increased, 2) the Michaelis constant (K_M) increased as well, but was not significant, 3) the energy of activation (E_{act}) was lower in adult muscle, 4) the HMM, α-helical content were not different and 5) also, no change in the content of histidine, tryptophan and tyrosine residues was noticed. The sulfhydryl (SH) group content was significantly reduced in adult animals (24 months) from 9.6 moles to 4.2 moles (Kaldor and Mim, 1975). The earlier work of Dow and Stracher (1971), also suggested that the differences between young and adult muscle myosin was due to the SH group content, which may influence the stability of the myosin molecule by conformational changes, rather than myosin ATPase changes. However, unlike the work of Kaldor and Mim, these authors reported greater content of SH groups in adult muscles, with a lesser sensitivity in terms of myosin ATPase activity.

Finally, another factor that confuses the developmental changes is the time discrepancies noted between onset of contractile properties and changes in contractile elements (e.g. myosin ATPase, light chains). The age dependent alterations in contractile properties indicates that the contraction times are altered by a

factor other than contractile elements. Cross-innervation studies which resulted in differentiation of muscle by contractile properties after 4 days, have shown that the contractile proteins are not altered until approximately 3 weeks. The factor which appears to influence the early developmental changes is the Ca^{++} uptake mechanism from the sarcoplasm reticulum (Ramirez and Pette, 1974). Also with cardiac tissue, the formation of various fractions of the sarcoplasm reticulum (e.g. t-tubes, longitudinal tubules) has been reported to occur at various time intervals from birth to 1 month (Schiebler et al., 1966). Thus, the differential nature of this development may well be associated with the muscles ability to produce a specific type of contraction, throughout the developmental phase. Pette et al., (1976) have shown that the sarcoplasm reticulum is the first muscular component to adapt to prolonged (8 hr/day) electrical stimulation (10 Hz), before metabolic and contractile proteins. This time related feature of adaptation appears to follow a logical pattern especially when the cellular events of muscular contraction are considered. Since the Ca^{++} uptake mechanism is the intermediate step between neural activity and binding of actin and myosin, it may well be the initial site of adaptation of the contractile apparatus.

4. Exercise and Sarcoplasmic Reticulum

The effects of physical training on fragmented sarcoplasm reticulum (FSR) calcium binding (storage, accumulation) and calcium uptake, from skeletal muscle, is uncertain. Barnard et al. (1970a) have reported no significant differences for trained (combination continuous and interval work) guinea pig gastrocnemius and plantaris muscle after both 9 and 18 weeks of training. Results from cardiac tissue FSR following swim training (150 min/day, 5 days/week) for 7-8 weeks have indicated a 25% and 29% increase in calcium storage and calcium uptake, respectively (Penpargkul et al., 1977). In contrast, hearts from dogs exercised for 8-10 weeks with a progressive, motor-driven treadmill program had not enhanced their ability for FSR calcium storage and/or uptake (Sordahl et al., 1977). The species difference, as well as the variety of exercise programs have been identified as possible factors for the discrepancies observed with cardiac tissue (Penpargkul et al., 1977; Tibbits et al., 1978).

The ability of the FSR to adapt to chronic stress (e.g. cross-innervation and electrical stimulation) have been reported. Cross-innervation of cat soleus and flexor digitorum muscles indicated that the calcium uptake could be altered, so that fast muscle resembled slow muscle after six months (Mommaerts et al., 1969). Electrically stimulated (8 hr/day, 10 impulses/sec) fast rabbit tibialis anterior showed electrophoretic patterns of

SR protein similar in content to the normal adult soleus (Ramirez et al., 1974). Thus, the results of trained and chronically altered FSR calcium binding and uptake, suggest that these components are adaptable, however the different training programs, animals and tissue selection confuse the extent of the adaptation.

5. Exercise and Contractile Proteins

Alterations of contractile characteristics have not only been observed with cross-innervation and post-natal development, but also their dependence on muscle function has been considered. Since soleus muscle serves a primary role in postural or antigravity function, it has been postulated that the disappearance of FG and FOG fibers, present at birth in soleus, may be attributed to their minimal involvement with postural function. The neural activity accompanying different types of function may be the primary reason for influencing characteristics of a particular fiber type (Pelloni-Mueller et al., 1976; Lewis et al., 1974). In an attempt to further observe this "function-fiber type" theory, exercise programs of varying intensities and duration have been employed. The contractile properties (e.g. time to peak tension, half relaxation, twitch tension and twitch to tetanus ratio) of trained guinea pig, gastrocnemius-plantaris muscles (N=5) over a twenty week period of treadmill running, did not differ significantly from sedentary controls (N=5) (Barnard et al., 1970b). Similar

observations on the tibialis anterior muscle for both endurance (N=4) and sprint (N=6) trained groups, have been reported after a seven month training program, when compared to control animals (N=3) (Fitts et al., 1973). With an intense swimming program (5-6 hours/day), the EDL muscle of young, male rats (20-25 days) exhibited a 9% decrease in time to peak tension and a 12% decrease in half contraction time (N=7) (Gutmann and Hajek, 1971). Due to the small number of animals (N) per group, the effects of training on contractile properties as evidenced by a number of investigators (Fitts et al., 1973; Gutmann and Hajek, 1971; Barnard et al., 1970), appears to be in doubt. Since these authors have used such widely different training protocols, in terms of intensity and duration, it is difficult to make any specific conclusions. Also the age and species of animal used in the experiments, confuses the interpretation of results. Generally, it would seem that age is important, as well as intensity in affecting contractile property changes with exercise and not the duraction of the training program.

In terms of myosin ATPase activity, the data are also controversial. Increased activities (18%) for rat EDL muscle after an intense swimming program was reported (Gutmann and Hajek, 1971). Similar results for young (20 days) rat soleus muscle after an eight week intense swimming program were reported (Syrovey et al., 1972). Also, when allowed to swim to exhaustion, every other day for 6 to 10 weeks, with 5% of body weight added, significant increases ($p \leq 0.01$) occurred for rat gastrocnemius myosin ATPase activities.

The values at 6 and 10 weeks of training were 44.58 $\pm$ 2.17 and 44.58 $\pm$ 1.52 ugPi/mg/myosin/5 minute (Wilkerson and Evonuk, 1971), which suggests that duration of the training is not critical beyond a certain period. Intensity of training, on the other hand, appears to be important, since less intense swimming programs (i.e. 30 min/day, no added weight) for 6 and/or 10 weeks produced no significant differences (Wilkerson and Evonuk, 1971). Endurance type treadmill running (2 hr. daily at 1.2 mph, 15% grade, 5 days/week) produced no significant changes in rat FG muscle, but a significant ($p \leq 0.01$) decrease (20%) for FOG muscle and a significant ($p \leq 0.05$) increase 20% for SO muscle was reported (Baldwin et al., 1975). The results of this particular study suggest that a "function-fiber type" relationship was occuring. Cardiac muscle myosin ATPase also increased significantly after an intense swimming program (Bhan and Scherier, 1975). Thus, it appears that enhanced muscle function via exercise, which also requires increased, specific nerve traffic to a muscle, may be important in producing changes of myosin ATPase activity. Also the quality of exercise (i.e. intensity) and not necessarily the quantity (e.g. duration), especially after a certain period, may be governing the adaptation of myosin ATPase. In addition, it seems that the stage of maturation of the neuro-muscular system at birth and through post-natal development will also affect myosin ATPase activity (Gutmann et al., 1974; Gutmann and Syrovy, 1967; Close, 1964).

In summary, the enzymatic (ATPase) changes associated with endurance training appear to be "function-fiber type" related, however, the effects of sprint training on myosin ATPase activity is obscure. Since the enzymatic activity of the contractile proteins seems to be structurally related (i.e. light chain components, SH group content), the adaptations from training should also be manifested by alterations at the molecular level of myosin. Also the effect of age or developmental level of the animal may be an important factor in determining whether or not a training regimen after birth will be of benefit, in terms of specific muscle fiber development, in an adult animal.

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APPENDIX C

DETAILS OF ASSAYS

A. Preparation of Myofibrils (Perry and Corsi, 1958)

Muscle samples were homogenized in borate-KCl buffer (pH 7.1): 0.039M sodium borate, 0.025 M KCl, 5 mM EDTA. Each of the samples were brought up to 5 ml and 1.0 ml of this homogenate used for total protein determination. The remaining homogenate was centrifuged at 800 x g for 15 minutes and the supernatant was discarded. The myofibril pellet was then washed and isolated as follows: a) resuspended in 20 vol. KCl-borate-EDTA buffer, b) centrifuged at 800 x g for 15 min., c) the precipitate was resuspended in 20 vol. borate-KCL, d) suspension was centrifuged at 800 x g for 15 min., e) the myofibril pellet was resuspended in the buffer without the 5 mM EDTA and two aliquots (1.0 ml) were used for protein determination and ATPase activities, f) the pellet was centrifuged at 800 x g for 15 min., g) the myofibrils were resuspended (4 vol.) in 50% glycerol/50%, 5 mM EDTA (pH 7.1) and stored at 0°C for 24 hours (Rome et al., 1967), and h) the glycinerated myofibrils were centrifuged at 800 x g for 15 min. and resuspended in 50% glycerol made fresh without the EDTA and stored for 10 days at -20°C.

The aliquots removed for protein determination and myofibrillar ATPase activities were treated as follows:

B. Lowry et al., (1951) Protein Determination

Reagents and Chemicals:

1. 0.5% cupric sulfate ($CuSo_4.5H_2O$)
2. 1.0% sodium potassium tartrate ($NaKC_4H_4O_6$)
3. 2.0% sodium carbonate (Na_2CO_3) - pH to 12.5 with 10 N NaOH
4. Lowry solution C: 61.7% $NaCO_3$

   35.7% Distilled Water

   1.3% $CuSO_4$

   1.3% $NaKC_4H_4O_6$

   100%
5. Folin Reagent - 1 to 1 (V/V) with distilled water.

Standard Curve: (50 mg Bovine Serum Albumin in 10 mls Buffer - 5 mg/ml).

Stock (ml)	Buffer (ml)	Concentration (mg/ml)	Final Concentration (ug)
1.0	0	5	30
0.8	0.2	4	25
0.6	0.4	3	19
0.4	0.6	2	12
0.2	0.8	1	6
0.1	0.9	0.5	3

Procedure:

a) Solubilizing Protein

   i) take 0.5 ml of homogenate or standard solution

   ii) add 1.0 ml of 0.3N KOH

   iii) warm in water both at 37°C for 30 minutes.

B (con't)

b) Reaction Mixture

i) take 0.1 ml of soluble protein solution from above

ii) add 5.0 ml of freshly prepared Lowry C

iii) add 0.3 ml of folin reagent while mixing in vortex hand mixer

iv) let this reaction mixture stand for 2 to 24 hours before analysis (used 18 hours).

c) Spectrophotometric Analysis

i) set wave length at 750 nm.

ii) prepare blank with 0.1 ml distilled water

iii) measure sample and record optical density.

C. Myofibrillar ATPase Activity (Baldwin et al., 1975)

Reagents and Chemicals:

1. Wash solution: 50 mM Tris, 5 mM $MgCl_2$, 100 mM KCl and 0.1% Triton X-100 at pH 7.4.
2. Suspension medium: 0.15 M KCl, 50 mM Tris (pH 7.4).
3. Incubation medium: 7 mM KCl, 0.01 mM $CaCl_2$, 1 mM $MgCl_2$ 20 mM Tris-HCl, 5 mM ATP.
4. 5.8N $HClO_3$
5. 1N KOH

Procedure

a) The myofibrils were washed (2X) with the wash solution and centrifuged at 800 x g for 15 minutes.

b) The pellet was resuspended in the wash solution and incubated at 4°C for 45 minutes.

c) After centrifugation (800 x g - 15 minutes), the precipitate was suspended in 0.15 M KCl - 50 mM Tris (pH 7.1).

d) Add 0.4 mg/ml of protein to incubation medium (without ATP), after pre-incubation for 5 minutes at 30°C in a metabolic shaker (2X).

e) After pre-incubation add 5 mM ATP to two vials.

f) Add 0.1 vol. 5.8N $HClO_3$ to one vial (control).

g) After addition of ATP to start reaction, allow to continue for 5 minutes. Add 0.1 vol. of 5.8N $HClO_3$ to other vial.

h) Add 7 vol. of 1N KOH to both vials.

i) Centrifuge medium (1000 x g - 15 minutes) to remove protein.

D. Determination of Inorganic Phosphate (Gawehn, 1974)

Reagents and Chemicals:

1. Phosphate standard (1 mM) - KH_2PO_4, potassium dihydrogen phosphate
2. 50 mM Tris buffer (pH 7.0)
3. Ammonium molybdate (2.5% w/v)
4. Reducing solution (1% w/v, 4-methylaminophenol sulphate, 2.7% w/v, sodium disulphite)
5. Phosphate reagent: 80 ml distilled water, 20 ml 5 N H_2SO_4, 20 ml ammonium molybdate and 20 ml reducing solution.

Standard Curve:

1. Dilute phosphate standard to 3 ml with distilled water.

Phosphate Solution (ml)	Water (ml)	Concentration (uMole)
0.4	2.6	0.4
0.6	2.4	0.6
0.8	2.2	0.8
1.0	2.0	1.0

2. Add 7 ml of phosphate reagent and allow to stand for 10 minutes.

Procedure:

a) Pipette into a test tube:

Tris buffer	2.40 ml
Sample/Standard	0.50 ml
Distilled water	0.10 ml
Phosphate reagent	7.00 ml

D (con't)

b) Mix and allow to stand at room temperature for 10 minutes.

c) Set the wave length at 578 nm and read samples against a blank containing distilled water instead of sample.

E. Preparation of Fragmented Sarcoplasmic Reticulum (Harigaya and Schwartz, 1969)

Reagents and Chemicals:

1. Homogenizing buffer: 10 mM sodium bicarbonate and 5 mM sodium azide (pH 7.0)
2. Wash buffer: 20 mM Tris-maleate and 0.6 M potassium chloride (pH 6.8)
3. Suspension medium: 20 mM Tris-maleate and 50 mM potassium chloride (pH 6.8)

Procedure:

a) The tissue was homogenized in 5 vol. of the homogenizing buffer.

b) The homogenate was centrifuged at 8,700 x g for 20 minutes.

c) The supernatant was centrifuged at 15,000 x g for 20 minutes.

d) Centrifuge the resulting supernatant for 30 minutes at 37,000 x g.

e) Suspend the precipitate in 5 vol. of the wash solution and centrifuge at 37,000 x g for 20 minutes.

f) Discard supernatant and resuspend precipate in small volume (1.5 ml) of the suspension medium and remove an aliquot (o.1 ml) for protein determination.

F. Calcium Binding

Reagents and Chemicals:

1. Incubation medium: 100 mM potassium chloride, 10 mM magnesium chloride, 20 mM Tris-maleate, 2 mM Tris-ATP and 0.01 mM calcium chloride. Also 0.01 uCi/ml of an aqueous calcium chloride solution containing ^{45}Ca was added.

Procedure:

a) Incubate 100 - 200 ug of FSR protein/ml with the incubation medium without the 2 mM Tris-ATP at 37°C for 1 minute, in a final volume of 1 ml.

b) Incubate as stated in above, but with 2 mM Tris-ATP added.

c) Terminate the reactions by placing the incubating solution on a collecting tube which is under vacuum.

d) Take an aliquot (0.8 ml) of the incubating sample and add to 5 ml of Acquasol (New England Nuclear, Cat. No. NEF-934).

e) The ATP - Ca^{++} Binding was determined from the difference, with and without the Tris-ATP.

G. Calcium Uptake

Reagents and Chemicals:

1. Incubation Medium: 100 mM potassium chloride, 10 mM magnesium chloride, 20 mM Tris-maleate, 2 mM Tris-ATP, 0.1 mM calcium chloride and 5 mM sodium axalate. Also 0.01 uCi/ml of an acqueous calcium chloride solution containing $^{45}Ca^{++}$ was added.

Procedure:

a) Thirty to sixty ug of FSR protein was added to the incubation medium to a final volume of 1 ml. The medium, plus sample and 2 mM Tris-ATP were incubated at 37°C for 30 minutes

b) Following incubation the medium was placed on Millipore filters, connected to a vacuum pump.

c) An aliquot (0.8 ml) of the medium was added to 5 ml of Acquasol and counted on a Phillips liquid scintillation counter.

H. Standard Curve Preparation

Reagents and Chemicals:

1. Stock 1000 uCi/ml or 92 ug Ca^{++}/ml or 0.001219 M
2. Working Stock: dilute 0.005 ml of stock to 5 ml with distilled water. (1 uCi/ml or 1.219 uM Ca^{++}/ml).
3. Incubation Mediums: as described for the calcium binding or uptake.

Procedure:

a)

Working Stock (ml)	Incubation Medium (ml)	Ca^{++} (uM)
0.00	0.800	0.0366
0.03	0.770	0.0366
0.02	0.780	0.0244
0.01	0.790	0.0122
0.005	0.795	0.0006

b) Add each of the standards to 5 ml of Acquasol and count in Phillips liquid scintillation counter.

I. NADH - Diaphorase (Dubowitz and Brooke, 1973)

Reagents and Chemicals:

1. Prepare 0.2 M Tris buffer (pH 7.4)
2. Nitro blue tetrazolium
3. NADH
4. Ethanol denatured (85% ethanol: 15% methanol)
5. Xylene
6. Diatex

Procedure:

a) Incubate 10u tissue sections for 30 minutes at 37°C in the following solution:

0.2 M Tris buffer (pH 7.4)	50 ml
nitro blue tetrazolium	50 mg
NADH	40 mg

- pH adjusted to 7.4

b) Dry sections in ascending alcohols (30%, 60% and 90%).

c) Clear the tissue in xylene and mount in diatex.

J. Myofibrillar ATPase (Guth and Samaha, 1969)

Reagents and Chemicals:

1. Prepare 0.1 M 2-amino-2-methyl-1-propanol with 0.018 M calcium chloride
2. Prepare 0.1 M Tris-HCl with 0.018 M calcium chloride
3. Prepare 0.07 M calcium chloride
4. Prepare 2% cobalt chloride
5. Prepare 0.1 M 2-amino-2-methyl-1-propanol
6. Prepare 1% ammonium sulfide
7. ATP
8. Ethanol denatured (85% ethanol: 15% methanol)
9. Xylene
10. Diatex

Procedure:

a) Alkaline preincubation

Preincubate 10u sections for 15 minutes in a solution of 0.1 M 2-amino-2-methyl-1-propanol containing 18 mM $CaCl_2$, adjusted to pH 10.4.

b) Rinse in 2 changes (1 minute each, with agitation) of 0.1 M Tris-HCl containing 18 mM $CaCl_2$, adjusted to pH 7.8. Drain off excess solution on blotting paper.

c) Incubation

Incubate sections at 37°C for 45 minutes in a 0.1 M 2-amino-2-methyl-1-propanol buffer containing 18 mM $CaCl_2$ and 2.7 mM ATP, adjusted to pH 9.4.

J.(con't)

d) Wash sections in 3 x 30 second changes of 0.07 M $CaCl_2$. Drain off excess solution.

e) Place in 2% cobalt chloride for 3 minutes.

f) Rinse in 4 x 30 second changes of 0.1 M 2-amino-2-methyl-1-propanol buffer (adjusted to pH 9.4).

g) Place in 1% yellow ammonium sulfide for 3 minutes.

h) Rinse in tap water for 3 to 5 minutes.

i) Dehydrate in ascending alcohols, clear in xylene, mount in diatex.

APPENDIX D

ANOVA SUMMARY TABLES

In this section following each ANOVA SUMMARY TABLE, the results of the Student-Newman-Keuls post-hoc test are given. In all tables the letter "a" refers to a significant difference between groups at the 0.05 level. In all tables the following abbreviations were used:

Group I	10d control
Group II	21d control
Group III	51d control
Group IV	10d sprint
Group V	21d sprint
Group VI	51d sprint
Group VII	10d endurance
Group VIII	21d endurance
Group IX	51d endurance

SA	Age Effect
SB	Exercise Effect
SAB	Interaction Effect
SE	Error

Analysis of Variance Table for Body Weights

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.320429E+06	2.	.160215E+06	454.507707	.000000
SB	.239478E+04	2.	.119739E+04	3.396839	.042272
SAB	.625759E+04	4.	.156440E+04	4.437994	.004148
SE	.158626E+05	45.	.352501E+03		
Homogeneity of Variance Test		.535564E+01			.000000

Results of Students-Newman-Keuls Test Applied to Body Weight Data

Group	Mean	IV	I	VII	II	V	VIII	VI	IX	III
		18.5	19.6	22.4	53.4	55.8	56.1	178.1	184.3	227.1
IV	18.5	--	1.1 (22.9)	3.9 (25.4)	34.9a (30.4)	37.3a (31.0)	37.6a (32.4)	159.6a (33.7)	165.8a (34.7)	208.6a (34.2)
I	19.6		--	2.8 (22.2)	33.8a (28.8)	36.2a (30.4)	36.5a (32.4)	158.5a (33.9)	164.7a (35.2)	207.5a (35.1)
VII	22.4			--	31.0a (22.2)	33.4a (25.4)	33.7a (28.0)	155.7a (29.8)	161.9a (31.2)	204.7a (31.1)
II	53.4				--	2.4 (22.9)	2.7 (27.6)	124.7a (30.4)	130.9a (32.4)	173.7a (32.8)
V	55.8					--	0.3 (21.9)	122.3a (26.4)	128.5a (29.1)	171.3a (29.8)
VIII	56.1						--	122.0a (21.9)	128.2a (26.4)	171.0a (28.0)
VI	178.1							--	6.2 (21.9)	49.0a (25.4)
IX	184.3								--	42.8a (21.9)
III	227.1									

Analysis of Variance Table for Soleus Muscle Weights

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.569660E+05	2.	.284880E+05	167.842687	.000000
SB	.015875E+03	2.	.529373E+02	.311946	.733592
SAB	.954877E+02	4.	.238719E+02	.140671	.966180
SE	.763652E+04	45.	.169700E+03		
Homogeneity of Variance Test		.512555E+02			.00000

Results of Student-Newman-Keuls Test Applied to Soleus Muscle Weights

Group	Mean	IV	I	VII	V	II	VIII	VI	IX	III
		5.8	8.4	9.1	21.2	21.4	23.8	79.0	81.3	85.7
IV	5.8	--	2.6 (15.9)	3.3 (17.6)	15.4 (20.1)	15.6 (22.5)	18.0 (22.5)	73.2a (22.5)	75.5a (24.0)	79.9a (23.7)
I	8.4		--	0.7 (15.4)	12.8 (19.1)	13.0 (22.0)	15.4 (22.5)	70.6a (23.5)	72.9a (24.4)	77.3a (24.3)
VII	9.1			--	12.1 (14.6)	12.3 (18.5)	14.7 (19.4)	69.9a (20.7)	72.2a (21.7)	76.6a (21.6)
V	21.2				--	0.2 (15.9)	2.6 (18.3)	57.8a (20.1)	60.1a (21.5)	64.5a (21.7)
II	21.4					--	2.4 (15.9)	57.6a (19.1)	59.9a (21.1)	64.3a (21.7)
VIII	23.8						--	55.2a (15.2)	57.5a (17.6)	61.9a (19.4)
VI	79.0							--	2.3 (15.2)	6.7 (17.6)
IX	81.3								--	4.4 (14.6)
III	85.7									--

Analysis of Variance Table for Plantaris Muscle Weights

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.201921E+06	2.	.100960E+06	351.756211	.000000
SB	.109004E+04	2.	.545021E+03	1.898907	.161537
SAB	.338166E+04	4.	.845414E+03	2.945506	.030270
SE	.129158E+05	45.	.287018E+03		
Homogeneity of Variance Test	.522217E+02				.00000

Results of Student-Newman-Keuls Test Applied to Plantaris Muscle Weights

Group	Mean	IV	I	VII	II	V	VIII	IX	VI	III
		8.2	10.0	12.3	30.6	31.7	33.5	130.3	145.7	168.1
			1.8	4.1	22.4	23.5	25.3	122.1a	137.5a	159.9a
IV	8.2	--	(20.7)	(22.9)	(27.4)	(27.9)	(29.3)	(30.4)	(31.3)	(30.9)
				2.3	20.6	21.7	23.5	120.3a	135.7a	158.1a
I	10.0		--	(20.0)	(26.0)	(27.4)	(29.2)	(30.6)	(31.8)	(31.7)
					18.3	19.4	21.2	118.0a	133.4a	155.8a
VII	12.3			--	(20.0)	(22.9)	(25.3)	(26.9)	(28.2)	(28.1)
						1.1	2.9	99.7a	115.1a	137.5a
II	30.6				--	(20.7)	(24.9)	(27.4)	(29.2)	(29.6)
							1.8	98.6a	114.0a	136.4a
V	31.7					--	(19.8)	(23.8)	(26.2)	(26.9)
								96.8a	112.2a	134.6a
VIII	33.5						--	(19.8)	(23.8)	(25.3)
									15.4	37.8a
IX	130.3							--	(19.8)	(22.9)
										22.4
VI	145.7								--	(19.0)
III	168.1									--

Analysis of Variance Table for Total Protein Content of Plantaris

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.134761E+06	2.	.673803E+05	59.812994	.000000
SB	.492658E+04	2.	.246827E+04	2.186624	.124349
SAB	.243518E+05	4.	.608796E+04	5.404236	.001253
SE	.495667E+05	44.	.112652E+04		
Homogeneity of Variance Test		.738816E+02			.00000

Results of Student-Newman-Keuls Test Applied to Total Protein Content of Plantaris

Group	Mean	I	IV	VII	II	IX	VI	III	V	VIII
		171.1	173.7	182.0	233.5	265.6	278.9	298.2	309.2	319.2
I	171.1	--	2.6 (39.2)	10.9 (49.4)	62.4a (54.4)	94.5a (55.4)	107.8a (60.7)	127.1a (58.0)	138.1a (62.0)	148.1a (61.2)
IV	173.7		--	8.3 (41.0)	59.6a (49.4)	91.9a (52.0)	105.2a (58.0)	124.5a (55.9)	135.5a (60.2)	145.5a (59.7)
VII	182.0			--	51.5a (42.9)	83.6a (49.4)	96.9a (56.8)	116.2a (56.1)	127.2a (60.7)	137.2a (61.0)
II	233.5				--	32.1 (41.0)	45.4 (51.6)	64.7a (52.6)	85.7a (58.0)	85.7a (58.7)
IX	265.6					--	13.3 (41.0)	32.6 (45.4)	43.6 (52.0)	53.6a (53.4)
VI	278.9						--	19.3 (39.7)	30.3 (49.4)	40.3 (52.6)
III	298.2							--	11.0 (37.8)	21.0 (45.4)
V	309.2								--	10.0 (37.8)
VIII	319.2									--

Analysis of Variance Table for Myofibrillar Protein Content of Plantaris

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.565267E+04	2.	.282688E+04	3.470426	.039857
SB	.176681E+04	2.	.883407E+03	1.084727	.346860
SAB	.184359E+04	4.	.460898E+03	.565932	.688642
SE	.358338E+05	44.	.814405E+03		
Homogeneity of Variance Test		.555986E+02			.00000

Results of Student-Newman-Keuls Test Applied to Myofibrillar Protein Content of Plantaris

Group	Mean	I	VII	II	VIII	V	IV	III	VI	IX
		136.0	154.7	162.8	167.5	168.7	170.3	177.1	178.9	181.8
I	136.0	--	18.7 (34.9)	26.8 (42.0)	31.5 (42.6)	32.7 (47.1)	34.3 (49.3)	41.1 (49.3)	42.9 (55.2)	45.8 (54.0)
VII	154.7		--	8.1 (34.9)	12.8 (40.6)	14.0 (46.2)	15.6 (49.3)	22.4 (49.9)	24.2 (56.0)	27.1 (55.2)
II	162.8			--	4.7 (33.7)	5.9 (42.0)	7.5 (46.2)	14.3 (47.7)	16.1 (53.9)	19.0 (53.6)
VIII	167.5				--	1.2 (32.1)	2.8 (38.6)	9.6 (40.8)	11.4 (47.7)	14.3 (47.5)
V	168.7					--	1.6 (33.3)	8.4 (38.6)	10.2 (46.2)	13.1 (47.1)
IV	170.3						--	6.8 (32.1)	8.6 (42.0)	11.5 (44.2)
III	177.1							--	1.8 (33.7)	4.7 (38.6)
VI	178.9								--	2.9 (34.9)
IX	181.8									--

Analysis of Variance Table for Myofibrillar ATPase Activities of Plantaris

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.305696E+01	2.	.152848E+01	745.688084	.000000
SB	.484477E-01	2.	.242289E-01	11.817914	.000078
SAB	.502761E-01	4.	.125690E-01	6.131955	.000519
SE	.901894E-01	44.	.204976E-02		
Homogeneity of Variance Test	.137394E+02				.08882

Results of Student-Newman-Keuls Test Applied to Myofibrillar ATPase Activities of Plantaris Muscle

Group	Mean	IV	VII	I	IX	VIII	III	II	V	VI
		0.187	0.188	0.191	0.585	0.701	0.719	0.720	0.748	0.755
IV	0.187	--	.001 (.056)	.004 (.064)	.398a (.071)	.514a (.079)	.532a (.076)	.533a (.086)	.561a (.085)	.568a (.091)
VII	0.188		--	.003 (.056)	.397a (.067)	.513a (.072)	.531a (.077)	.532a (.087)	.560a (.086)	.567a (.093)
I	0.191			--	.394a (.054)	.510a (.062)	.528a (.068)	.529a (.079)	.557a (.079)	.564 (.086)
IX	0.585				--	.116a (.052)	.134a (.062)	.135a (.074)	.163a (.076)	.170a (.087)
VIII	0.701					--	.018 (.050)	.109 (.065)	.047 (.068)	.054 (.077)
III	0.719						--	.001 (.054)	.029 (.062)	.036 (.072)
II	0.720							--	.028 (.056)	.035 (.070)
V	0.748								--	.007 (.056)
VI	0.755									--

Analysis of Variance Table for Total Protein Content of Soleus

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.157996E+02	2.	.789979E+01	.003024	.996981
SB	.712680E+04	2.	.356315E+04	1.368934	.265797
SAB	.332478E+05	4.	.831194E+04	3.181719	.021737
SE	.120171E+05	46.	.261241E+04		
Homogeneity of Variance Test	.113136E+03				.00000

Results of Student-Newman-Keuls Test Applied to Total Protein Content of Soleus Muscle

Group	Mean	VIII	IX	I	V	IV	III	VII	VI	II
		209.4	224.5	237.1	242.2	242.6	244.3	271.1	282.2	302.8
VIII	209.4	--	15.1 (57.6)	27.7 (69.2)	32.8 (76.3)	33.3 (81.3)	34.9 (81.7)	61.7 (88.4)	72.8 (91.0)	93.4 (97.9)
IX	224.5		--	12.6 (59.8)	17.7 (71.9)	18.1 (79.2)	19.8 (85.5)	46.6 (88.4)	57.7 (91.8)	78.3 (98.9)
I	237.5			--	4.7 (59.8)	5.1 (71.9)	6.8 (76.3)	33.6 (84.4)	44.7 (88.4)	65.3 (96.0)
V	242.2				--	0.4 (59.8)	2.1 (69.2)	28.9 (79.2)	40.0 (84.4)	60.6 (92.5)
IV	242.6					--	1.7 (57.6)	28.5 (71.9)	39.6 (79.2)	60.2 (88.3)
III	244.3						--	26.8 (57.6)	37.9 (69.2)	58.5 (80.2)
VII	271.1							--	11.1 (59.8)	31.7 (75.2)
VI	282.2								--	20.6 (62.5)
II	302.8									--

Analysis of Variance Table for Myofibrillar Protein Content of Soleus

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.102551E-01	2.	.512754E-02	4.019066	.024622
SB	.133521E+00	2.	.667607E-01	52.328341	.000000
SAB	.780095E-01	4.	.195024E-01	15.286330	.000000
SE	.586870E-01	46.	.127580E-02		
Homogeneity of Variance Test	.420381E+02				.00000

Results of Student-Newman-Keuls Test Applied to Myofibrillar ATPase Activities of Soleus Muscle

Group	Mean	VII 0.172	IV 0.193	II 0.307	I 0.312	III 0.316	V 0.318	VI 0.323	VIII 0.339	IX 0.378
VII	0.172	--	.021 (.043)	.135a (.049)	.140a (.054)	.144a (.058)	.146a (.060)	.151a (.063)	.167a (.064)	.206a (.066)
IV	0.193		--	.114a (.044)	.119a (.053)	.123a (.059)	.125a (.062)	.130a (.063)	.147a (.068)	.185a (.070)
II	0.307			--	.005 (.044)	.009 (.051)	.011 (.056)	.016 (.058)	.033 (.063)	.071a (.065)
I	0.312				--	.004 (.042)	.006 (.051)	.011 (.054)	.028 (.060)	.066a (.063)
III	0.316					--	.002 (.042)	.007 (.049)	.024 (.056)	.062a (.060)
V	0.318						--	.005 (.041)	.022 (.051)	.060a (.056)
VI	0.323							--	.016 (.041)	.055a (.049)
VIII	0.339								--	.039a (.042)
IX	0.378									--

Analysis of Variance Table for Myofibrillar ATPase Activities of Soleus

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.214933E+04	1.	.214933E+04	1.846287	.191973
SB	.128038E+05	2.	.640189E+04	5.499252	.014394
SAB	.999481E+04	2.	.499740E+04	4.292792	.030964
SE	.197904E+05	17.	.116414E+04		
Homogeneity of Variance Test		.118209E+02			.03716

Results of Student-Newman-Keuls Test Applied to Myofibrillar Protein Content of Soleus

Group	Mean	II	VIII	IX	III	VI	V
		124.7	143.5	180.8	184.0	189.2	226.3
II	124.7	--	18.8 (54.9)	56.1 (66.7)	59.3 (65.0)	64.5 (69.6)	101.6a (83.2)
VII	143.5		--	37.3 (58.7)	40.5 (63.8)	45.7 (70.8)	82.8 (84.7)
IX	180.8			--	3.2 (52.5)	8.4 (55.5)	45.5 (70.8)
III	184.0				--	5.2 (52.5)	42.3 (71.5)
VI	189.2					--	37.1 (52.5)
V	226.3						--

Analysis of Variance Table for Fragmented Sarcoplasmic Reticulum Yield of Plantaris Muscle

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.241429E+02	1.	.241429E+02	33.351606	.000037
SB	.123310E+01	2.	.616548E+00	.851717	.446315
SAB	.633095E+00	2.	.316548E+00	.437288	.653760
SE	.108583E+02	15.	.723889E+00		
Homogeneity of Variance Test			.526751E+00		.993710

Results of Student-Newman-Keuls Test Applied to Fragmented Sarcoplasmic Reticulum Yield to Soleus Muscle

Group	Mean	III	VI	IX	VII	IV	I
		5.7	5.8	7.0	7.9	8.3	8.4
III	5.7	--	0.1 (2.4)	1.3 (1.9)	2.2a (1.6)	2.6a (1.5)	2.7a (1.4)
VI	5.8		--	1.2 (2.4)	2.1a (1.8)	2.5a (1.6)	2.6a (1.5)
IX	7.0			--	0.9 (2.2)	1.3 (1.8)	1.4 (1.6)
VII	7.9				--	0.4 (2.1)	0.5 (1.7)
IV	8.3					--	0.1 (2.1)
I	8.4						--

Analysis of Variance Table for Calcium Binding Ability of Plantaris Muscle

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.841509E+03	1.	.841509E+03	90.704277	.000000
SB	.433800E+02	2.	.216900E+02	2.337914	.130673
SAB	.261343E+02	2.	.130671E+02	1.408477	.275064
SE	.139163E+03	15.	.927750E+01		
Homogeneity of Variance Test		.296306E+01			.798290

Results of Student-Newman-Keuls Test Applied to Calcium Binding Ability of Plantaris Muscle

Group	Mean	IV	I	VII	III	VI	IX
		9.5	10.3	11.3	20.0	23.5	26.1
IV	9.5	--	0.8 (6.9)	1.8 (5.6)	10.5a (5.4)	14.0a (5.0)	16.6a (4.8)
I	10.3		--	1.0 (6.9)	9.7a (6.1)	13.2a (5.4)	15.8a (5.0)
VII	11.3			--	8.7a (7.5)	12.2a (6.1)	15.8a (5.4)
III	20.0				--	3.5 (6.9)	6.1a (5.6)
VI	23.5					--	2.6 (6.9)
IX	26.1						--

Analysis of Variance Table for Calcium Uptake Activity of Plantaris Muscle

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.188381E-02	1.	.188381E-02	49.135840	.000004
SB	.681746E-05	2.	.340873E-05	.088911	.915406
SAB	.531032E-04	2.	.265516E-04	.692550	.515604
SE	.575083E-03	15.	.383389E-04		
Homogeneity of Variance Test	.528409E+00				.993670

Results of Student-Newman-Keuls Test Applied to Calcium Uptake Activity of Plantaris Muscle

Group	Mean	IV	VII	I	III	IX	VI
		0.145	0.146	0.149	0.164	0.165	0.168
IV	0.145	--	0.001 (0.014)	0.004 (0.011)	0.019a (0.011)	0.020a (0.010)	0.023a (0.010)
VII	0.146		--	0.003 (0.014)	0.018a (0.012)	0.019a (0.011)	0.022a (0.010)
I	0.149			--	0.015a (0.015)	0.016a (0.012)	0.019a (0.011)
III	0.164				--	0.001 (0.016)	0.004 (0.013)
IX	0.165					--	0.003 (0.016)
VI	0.168						--

Analysis of Variance Table for Fragmented Sarcoplasmic Reticulum of Soleus Muscle

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.200604E+02	1.	.200604E+02	24.548673	.000173
SB	.733571E+00	2.	.366786E+00	.448851	.646661
SAB	.332214E+01	2.	.166107E+01	2.032720	.165513
SE	.122575E+02	15.	.817167E+00		
Homogeneity of Variance Test		.448591E+01			.649200

Results of Student-Newman-Keuls Test Applied to Fragmented Sarcoplasmic Reticulum Yield of Plantaris Muscle

Group	Mean	IV	I	VII	VI	IX	III
		4.3	4.4	4.6	6.0	6.8	6.9
IV	4.3	--	0.1 (1.9)	0.3 (1.6)	1.7a (1.5)	2.5a (1.4)	2.6a (1.3)
I	4.4		--	0.2 (1.9)	1.6 (1.7)	2.4a (1.5)	2.5a (1.4)
VII	4.6			--	1.4 (2.1)	2.2a (1.7)	2.3a (1.5)
VI	6.0				--	0.8 (2.2)	0.9 (1.8)
IX	6.8					--	0.1 (2.2)
III	6.9						--

Analysis of Variance Table for Calcium Binding Ability of Soleus Muscle

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.272583E+04	1.	.272583E+04	85.587276	.000000
SB	.195103E+01	2.	.975516E+00	.030630	.969895
SAB	.139494E+01	2.	.697421E+00	.021898	.978371
SE	.477728E+03	15.	.3/8486E+02		
Homogeneity of Variance Test		.164676E+02			.005620

Results of Student-Newman-Keuls Test Applied to Calcium Binding Ability of Soleus Muscle

Group	Mean	VI 6.3	IX 6.4	III 6.7	VII 28.7	IV 29.8	I 29.9
VI	6.3	--	0.1 (14.8)	0.4 (12.0)	22.4a (10.0)	23.5a (9.3)	23.6a (8.8)
IX	6.4		--	0.3 (14.8)	22.3a (11.2)	23.4a (10.0)	23.5a (9.3)
III	6.7			--	22.0a (13.8)	23.1a (11.2)	23.2a (10.0)
VII	28.7				--	1.1 (12.8)	1.2 (10.4)
IV	29.8					--	0.1 (12.8)
I	29.9						--

Analysis of Variance Table for Calcium Uptake Activity of Soleus Muscle

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SA	.100321E-03	1.	.100321E-03	1.887910	.189623
SB	.360024E-03	2.	.180012E-03	3.387574	.061091
SAB	.413167E-03	2.	.206583E-03	3.887611	.043623
SE	.797083E-03	15.	.531389E-04		
Homogeneity of Variance Test		.148652E+01			.940430

Results of Student-Newman-Keuls Test Applied to Calcium Uptake Activity of Soleus Muscle

Group	Mean	IV	I	VII	III	IV	IX
		0.139	0.141	0.143	0.144	0.145	0.159
IV	0.139	--	0.002 (0.016)	0.004 (0.013)	0.005 (0.013)	0.006 (0.012)	0.020a (0.011)
I	0.141		--	0.002 (0.016)	0.003 (0.014)	0.004 (0.013)	0.018a (0.012)
VII	0.143			--	0.001 (0.018)	0.002 (0.014)	0.016a (0.013)
III	0.144				--	0.001 (0.019)	0.015 (0.016)
IV	0.145					--	0.014 (0.019)
IX	0.159						--

APPENDIX E

LABORATORY CHOW INGREDIENTS

PURINA

LABORATORY CHOW

GUARANTEED ANALYSIS

Crude protein not less than.....23.0%

Crude fat not less than..... 4.5%

Crude fiber not more than..... 6.0%

Ash not more than..... 8.0%

Added minerals not more than..... 2.5%

INGREDIENTS

Ground extruded corn, soybean meal, ground oat groats, dried beet pulp, wheat germ meal, fish meal, dehydrated alfalfa meal, dried milk products, cane molasses, meat and bone meal, brewers' dried yeast, wheat middlings, animal fat preserved with BHA, calcium carbonate, dicalcium phosphate, salt, calcium iodate, animal liver meal, vitamin B_{12} supplement, methionine hydroxy analogue calcium, calcium pantothenate, choline chloride, folic acid, thiamin, niacin, pyridoxine, ferrous sulfate, vitamin A supplement, vitamin E supplement, iron sulfate, iron oxide, manganous oxide, copper oxide, zinc oxide.

APPENDIX F

RAW DATA

Raw Data for Body and Muscle Weights

	10 days			21 days			51 days		
	Body (g)	Plantaris (mg)	Soleus (mg)	Body (g)	Plantaris (mg)	Soleus (mg)	Body (g)	Plantaris (mg)	Soleus (mg)
CONTROL	18.0	10.0	8.0	56.5	34.0	24.0	242.0	163.0	86.0
	17.0	10.0	10.0	56.0	34.0	24.0	225.0	189.0	100.0
	19.0	10.0	6.0	54.0	33.0	21.0	217.0	200.0	114.0
	16.0	5.0	8.0	45.0	31.0	22.0	235.0	161.0	75.0
	28.0	15.0	10.0	55.5	21.0	16.0	251.0	185.0	87.0
							207.0	128.0	67.0
							213.0	151.0	71.0
SPRINT	18.0	8.0	10.0	51.0	29.0	16.0	199.0	169.0	111.0
	18.0	6.0	4.0	49.0	38.0	20.0	201.5	165.0	102.0
	19.0	5.0	4.0	70.5	37.0	31.0	139.5	129.0	71.0
	20.0	10.0	5.0	70.0	36.0	27.0	127.5	124.0	53.0
	19.0	10.0	5.0	48.0	24.0	18.0	199.0	131.0	62.0
				46.5	26.0	15.0	202.0	156.0	75.0
ENDURANCE	19.0	10.0	6.0	51.5	26.0	29.0	207.0	150.0	113.0
	17.0	8.0	6.0	51.0	32.0	19.0	182.0	77.0	60.0
	21.0	11.0	10.0	71.0	40.0	30.0	201.0	165.0	102.0
	17.0	7.0	7.0	66.0	43.0	28.0	115.0	102.0	62.0
	16.0	6.0	5.0	49.0	31.0	18.0	196.0	130.0	89.0
	19.0	9.0	8.0	48.0	29.0	19.0	205.0	158.0	62.0
				48.0	35.0	22.0			

Raw Data for Plantaris Total Protein (mg/g), Myofibril Protein (mg/g) and Mg^{++} - ATPase Activities (umoles Pi/mg/min)

	10 days			21 days			51 days		
	Total	Myofibril	ATPase	Total	Myofibril	ATPase	Total	Myofibril	ATPase
CONTROL	173.0	138.8	.190	240.0	165.7	.735	289.3	202.5	.691
	174.5	138.0	.228	244.7	168.3	.643	275.4	220.3	.722
	173.5	136.0	.197	229.0	148.9	.644	322.8	216.3	.704
	173.5	140.4	.107	215.9	163.6	.803	369.3	162.6	.690
	167.1	133.3	.220	237.1	167.5	.776	280.7	159.0	.748
	165.2	130.0	.203				334.0	101.9	.763
							216.2	177.1	.715
SPRINT	178.9	176.0	.152	303.1	145.3	.759	227.6	174.1	.753
	172.8	170.0	.228	307.2	147.8	.800	295.6	189.2	.796
	176.2	171.7	.143	309.2	148.4	.748	268.0	206.4	.712
	172.8	170.0	.215	315.7	192.9	.741	305.9	205.3	.777
	170.8	167.0	.198	312.7	190.7	.768	297.2	119.6	.739
	170.8	167.0	.187	307.0	187.3	.673			
ENDURANCE	183.5	156.0	.190	310.9	239.4	.672	187.1	181.5	.629
	181.6	153.8	.191	337.7	219.5	.727	298.8	148.3	.534
	185.7	158.6	.178	381.7	147.4	.782	208.2	143.7	.562
	178.4	151.7	.210	248.3	167.0	.568	293.3	222.9	.607
	180.7	153.6	.168	286.4	125.4	.701	288.3	184.5	.581
				286.6	125.0	.705	317.8	209.8	.598
				383.0	148.0	.753			

Raw Data for Plantaris FSR Protein Yield (mg/g), ATP - Ca^{++} - Binding (nmoles Ca^{++}/mg) and ATP - Ca^{++} - Uptake (umoles Ca^{++}/mg)

	10 days			51 days		
Control	Yield	Binding	Uptake	Yield	Binding	Uptake
	4.1	8.7	.141	7.9	19.6	.170
	5.3	14.1	.149	6.6	22.0	.159
	4.7	7.1	.155	6.3	18.3	.163
	3.3	11.4	.151			
Sprint	4.7	8.7	.155	6.9	22.7	.173
	4.1	11.4	.139	4.8	19.9	.170
	3.3	7.1	.147	6.3	27.8	.162
	5.0	10.9	.141			
Endurance	3.3	11.4	.142	6.8	31.1	.168
	5.3	8.7	.155	7.4	21.7	.168
	4.7	14.1	.147	6.1	25.4	.160
	5.0	10.90	.139			

Raw Data for Soleus Total Protein (mg/g), Myofibril Protein (mg/g) and Mg^{++} - ATPase Activities (umoles Pi/mg/min)

	10 days			21 days			51 days		
	Total	Myofibril	ATPase	Total	Myofibril	ATPase	Total	Myofibril	ATPase
CONTROL	236.5		.313	199.8	85.4	.287	261.6	196.2	.194
	233.7		.306	199.6	85.1	.200	229.7	172.3	.204
	241.1		.333	379.1		.193	283.3	215.1	.178
	236.5		.306	365.9	163.5	.152	313.2	220.8	.199
	233.7		.303	369.4	164.7	.134	335.5	115.6	.201
	241.1		.312				287.2		.192
							283.0		.213
SPRINT	250.4		.299	240.7		.289	317.3	197.9	.330
	246.7		.280	232.9		.348	275.2	218.6	.347
	237.5		.307	236.4		.316	316.0	201.0	.338
	237.5		.308	252.5	161.1	.318	283.7		.339
	241.8		.325	252.4	145.5	.317	255.1		.361
	241.8		.325	238.3	123.9	.317	254.6	198.3	.322
ENDURANCE	276.1		.311	210.8		.361	265.9	175.5	.402
	268.2		.333	203.6		.303	200.2		.398
	277.0		.293	207.9		.323	199.1		.377
	275.2		.323	213.9	161.1	.327	243.6	185.6	.352
	267.1		.320	208.9	145.5	.301	214.0	178.4	.361
	263.0		.314	211.8	123.9	.322	224.0		.378
				208.9		.323			

Raw Data for Soleus FSR Protein Yield (mg/g), ATP - Ca^{++} - Binding (nmoles Ca^{++}/mg) and ATP - Ca^{++} - Uptake (umoles Ca^{++}/mg)

	10 days			51 days		
Control	Yield	Binding	Uptake	Yield	Binding	Uptake
	8.4	31.2	.142	6.5	6.4	.144
	9.3	39.1	.133	4.9	7.6	.149
	7.0	21.8	.151	5.8	6.0	.140
	8.7	27.6	.139			
Sprint	8.7	27.6	.146	5.5	6.8	.129
	6.8	22.7	.151	5.9	6.0	.147
	8.4	31.2	.139	6.1	7.0	.140
	9.3	37.6	.142			
Endurance	7.0	21.8	.146	7.2	6.3	.169
	6.8	22.7	.142	6.6	7.0	.151
	8.4	39.1	.133	7.3	5.9	.157
	9.3	31.2	.151			

APPENDIX G

OBSERVATIONS ON EXERCISING

10 DAY RATS

At 10 days of age rats are not strong enough to support their own body weight in a normal standing position. Any movement (e.g. walking or running) was accomplished with their ankles in a lateral flexed position. It is critical that the training programs start at a speed which the animals can handle. If set too fast the animals will not attempt to move with the treadmill surface, and simply be carried away. Once the training speeds were determined (Table 2:1), traditional motivational techniques (e.g. shock grid, air jets) had to be abandoned. The motivational technique from animal to animal varied, however, the majority of animals responded to slight pressure on the tail or posterior region. Constant attention is required, and it is recommended that only 1 animal exercise at any one time. The lack of endurance of 10 day animals required that their exercise programs be interrupted with short rest periods. It was observed that forcing the animal to keep running often resulted in injuries, and injured animals were usually neglected by the mother which resulted in either death or very retarded growth rates. Since rest periods were necessary, the time required to complete 10 minutes of exercise was normally between 15 - 30 minutes per animal.

The treadmill compartments were placed in such a manner that a space of approximately 0.25 inch existed between them and the running surface. Gloves were used at all times until the animals were weaned, so that a human scent would not be perceived by the mother. Finally, it should be noted that running programs

at this age are not characteristic of normal, adult running programs, in that exercise is performed on the ankles. This pattern of running may have a different effect on recruitment patterns, however, after 2-3 training sessions the animals were able to support their weight in a normal fashion. However, it was observed that these animals were exposed to a situation which interrupted normal developmental patterns of growing rats, and placed more stress on the neural-muscle-skeletal systems.

APPENDIX H

ATTRITION VALUES FOR TRAINING

YOUNG RATS

Following is a table which indicates the percentage (%) of animals lost with respect to each age and/or experimental group.

	AGE (days)		
GROUP	10	21	51
Control	5	29	22
Sprint	3	40	40
Endurance	1	40	40